RESPONSES OF ELODEA DENSA TO GROWTH-REGULATING SUBSTANCES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 555

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Introduction

Numerous studies on *Lemna* by CLARK and co-workers (6), an investigation of *Elodea* by SOLTYS, UMRATH, and UMRATH (23), and several studies on *Chlorella* and other algae, are some of the major investigations with growth-regulating substances using aquatic or submerged plants. The experiments described here are principally concerned with the gross responses of excised shoots and segments of *Elodea densa* when grown in a liquid nutrient medium containing growth substances.

MATERIAL AND METHODS.—Although the generic name *Anacharis* is employed by some writers, since *Elodea* has been proposed as a *nomen conservandum* by Dr. HAROLD ST. JOHN (14, p. 292), it is the one adopted in this study. The species of *Elodea* most commonly investigated has been the North American one, *Elodea canadensis* Michx. The species used in this investigation is *Elodea densa* (Planch.) Casp. (verified by C. A. WEATHERBY of the Gray Herbarium). *E. densa* is one of the largest species of the genus and is commonly grown in aquaria. It is South American, with a range from southern Brazil to the delta of the La Plata in Argentina (24).

Using the agar-block technique, HOMÈS and VAN SCHOOR (12) demonstrated that in *Elodea* auxins formed at the growing point inhibited bud growth as well as promoted internodal axial growth. SOLTYS *et al.* (23) studied the responses of various species of the genus to a series of dilutions of l-histidine compounds and indoleacetic acid. VAN OVERBEEK (18) has found auxin in a species of *Elodea*. CORMACK (8) studied the effects of ethylene on root-hair formation in *E. canadensis*.

The most complete account of the anatomy of species of this genus is that of CASPARY (5). Other such studies include the investigations of HOLM (11) and SIDDALL (21). Most of the published work on the anatomy of the species treats of the structure of the shoot apex, stem, and root, and the manner of flower formation. Few investigations have considered the anatomy of the bud-node region of the primary axis and the manner of root formation. CASPARY (5) shows diagrams of the bud node of *Elodea canadensis* but does not describe it in detail. A study of the structure of the bud node has been made here to clarify the responses of these segments to treatment with growth-regulating substances.

In *Elodea densa* the lateral buds are rather regular in their divergence from the primary axis, generally at every twelfth node. Since buds are not formed at every node, the term "bud node" is used to include that portion of the primary axis from which both leaves and a single axillary bud diverge. Excised bud nodes containing a single whorl of leaves, and longer bud-node segments containing three separate whorls, have been employed in the present experiments.

The roots are formed early in the development of the axis and the bud-node region. These roots may be found as small primordia within the cortical tissues of the primary axis, only a few millimeters from the shoot apex. At each bud node there are four dormant root primordia. Two are located in the primary axis in the region of divergence of the secondary axis (one either side of the secondary axis); two are located in the base of the secondary axis near the point of its divergence from the primary axis. CASPARY (5) has termed the undeveloped shoot axis (inclosed within the lateral bud) the secondary axis. These primordia remain inactive throughout the entire length of the shoot. In one shoot about 1 m. in length, a total of eighteen bud nodes was counted. Roots had appeared only at the first bud node at the base of the shoot. Since each of these bud nodes under certain conditions is capable of forming a complete new plant, the potential vegetative reproductive capacity of this species is evident.

In the present experiments the two primordia of the primary axis of the bud node upon emergence are termed the first and second roots; the two primordia of the secondary axis are termed the third and fourth roots. With certain types of treatment fifth roots may appear.

In rooted shoots growing in the culture tanks, generally neither root nor axillary bud growth occurs; the roots develop when a portion of the axis becomes broken or detached. They then develop at the most basal of the nodes of the segment. If the apical bud of the segment is intact, usually none of the axillary buds develop. If the shoot apex is damaged, however, then the bud next below begins to develop. Apical dominance is very evident, even in detached segments containing only two successive buds. In this case the distal bud will grow, while the proximal one will be inhibited and only roots will form.

The light source for most of these experiments consisted principally of 36-inch, 30-watt Mazda fluorescent daylight tubes. The various intensities were all used for a period of 13 hours each day. The intensities in foot candles were measured with a Weston photometer at the rim of the glass tumbler. From data supplied by Dr. ROHRBAUGH (20), the energy value for one of the commonly used intensities, 400 f.c., has been estimated to be 830×10^{-6} gm. cal./cm.^{2}/sec. (since 280 f.c. was equal to 581×10^{-6} gm. cal./cm.^{2}/sec.). ROHRBAUGH'S studies (19) include a review of the work on fluorescent light and plant growth.

The chemical name and the source of the principal substances employed are as follows: beta-(indole-3) acetic acid; beta-(indole-3) propionic acid; gamma-(indole-3)-n-butyric acid; alpha naphthalene acetic acid; phenylacetic acid; thiophenyl acetamide; l-tryptophane; glycine; nicotinic acid; ascorbic acid; and l-histidine monohydrochloride—all supplied by Eastman Kodak Co.; thiamin chloride (vitamin B_1) from Merck; alpha naphthalene acetamide from American Chemical Paint Co.; and calcium pantothenate from International Vitamin Corp.

Investigation

EXPERIMENT 1.—Preliminary experiments had demonstrated that excised bud nodes, with a single whorl of leaves and the axillary bud, were capable of developing into complete new plants. Entire shoots and excised bud nodes were found to grow for considerable time in aqueous solutions of growth-regulating substances under fluorescent light or daylight.

Indoleacetic, indolepropionic, indole-

butyric, phenylacetic, and naphthaleneacetic acids; l-tryptophane, and thiamin chloride were the substances used. Stock solutions were prepared by dissolving a small amount of the crystals in hot distilled water (except those substances readily soluble). After repeated heating to near boiling, and with continued shaking, the substances would dissolve. The solutions were then made to volume and stored in a refrigerator at 10° C. Culture solutions were prepared by adding to tap water a sufficient amount of the chemical to make its concentration 1 p.p.m. One series of cultures contained a mixture of vitamin B_1 and indolebutyric acid, each at 1 p.p.m. The slightly alkaline tap water (a recent analysis showed calcium 32 p.p.m., magnesium 11, potassium 1, nitrate 0.1, and sulphate 15) was used as a nutrient medium in most of the experiments. The cultures were not grown under sterile conditions. All experiments with fluorescent light were conducted in a basement room.

Bud nodes were cut from shoots of healthy and firmly rooted plants taken from the stock cultures maintained in tanks in the greenhouse. In cutting out the bud nodes, the size of the stump was determined by making the cut through the internode halfway between the bud node whorl and the next successive whorl. These excised nodes with but a single whorl of leaves measured about 1.0 cm. in length. Since these segments were somewhat variable, all for any given trial were cut at one time and thoroughly mixed, then picked at random and placed in glass tumblers in 200 ml. of solution. In experiment 1 there were placed in each glass three bud nodes and one stem segment (that is, the portion of the axis between two successive bud nodes). The length of such a segment may vary from 7 to 10 cm. It has no buds present, and hence does not usually regenerate. Each compound was tested in triplicate, and controls in tap water alone were tested in replicates of six. The cultures were all grown at an average intensity of about 300 f.c. Readings taken at several different times indicated a temperature range of 28°–30° C. The solutions were not changed during the course of this experiment, although tap water was added to replace that lost through evaporation. The experiment ran August 2–15, 1941.

Detailed observations were made on the cultures every 4 days for 16 days. Data were taken on the length of all roots formed, the number per node, the size of the bud, and general observations on root-hair formation (tables 1, 2). Here, as in the other experiments, the measurements given generally include the sum of the measurements of all the nodes in any one culture. The methods of measuring and tabulating of data in these experiments have been in part adapted from BROWN (4). In the two tables the various values of the two series of controls (two series each of three cultures) have been averaged for better comparison with the other cultures. The values in the tables of some experiments show a decrease over values for earlier growth periods. This is chiefly due to death of roots, loss of bud scales, etc.

In the duplicate series of controls in tap water, consisting of eighteen bud nodes, only three roots appeared at the end of the fourth day. The greatest number of roots appeared in the mixed B_1 and indolebutyric cultures, and root hairs were observed on one root 1.1 cm. in length. No growth of the buds occurred in the first 4 days.

In total root length, the indolepropionic cultures led all the series (except the mixed cultures) at both 12 and 16 days. At 16 days the second highest total

TABLE 1

TOTAL ROOT LENGTH, AVERAGE INDIVIDUAL ROOT LENGTH, AND TOTAL ROOT NUMBER OF NINE BUD NODES IN CULTURES AT 1 P.P.M. LENGTH VALUES IN CM.

CULTURE	GROWTH PERIOD											
	4 days			8 days			12 days			16 days		
	Root	Ave. root	Root no.	Root	Ave. root	Root no.	Root	Ave. root	Root no.	Root	Ave. root	Root no.
Indoleacetic	0.30	0.30	1	12.95	1.85	7	26.90	2.23	15	41.00	2.73	15
Indolepropionic	1.80	0.30	6	25.80	2.34	11	47.15	2.61	18	60.40	3.02	20
Indolebutyric	1.52	0.25	6	21.20	1.76	12	33.10	1.57	21	44.15	1.76	25
l-Tryptophane	2.57	0.51	5	20.60	2.94	7	33.45	3.34	10	33.95	3.39	11
Phenylacetic	0.30	0.30	1	7.75	1.29	6	19.15	2.38	8	31.75	2.88	11
Naphthaleneacetic	1.35	0.22	6	7.85	0.65	12	6.80	0.68	10	.....	.....	.....
Vitamin B_1	0.00	0.00	0	5.90	1.96	3	15.90	2.65	11	25.67	2.56	11
Vitamin B_1 and indolebutyric	3.56	0.44	8	27.95	1.55	18	39.25	1.57	25	41.45	1.73	28
Average of two control series	0.25	0.16	1.5	7.50	1.77	5.5	16.75	2.64	8	29.30	2.90	9

root length occurred in the indolebutyric cultures. Although the indolepropionic cultures were the highest in total root length, they did not have the greatest average root length; they stood second to tryptophane, which had the highest average root length throughout the entire 16-day period.

TABLE 2

TOTAL SHOOT LENGTH OF NINE BUD NODES IN CULTURES AT 1 P.P.M. VALUES IN CM.

CULTURE	GROWTH PERIOD		
	8 days	12 days	16 days
Indoleacetic	3.90	6.90	9.10
Indolepropionic	3.80	6.25	7.55
Indolebutyric	2.70	2.65	4.10
l-Tryptophane	5.40	7.50	9.35
Phenylacetic	5.40	6.80	8.60
Naphthaleneacetic	5.50	6.20	.....
Vitamin B_1	5.80	7.85	8.90
Vitamin B_1 and indolebutyric	2.95	2.25	3.70
Average of two control series	5.90	7.90	9.47

The greatest number of roots occurred in the mixed cultures throughout the 16 days. At 16 days this was three times the numbers formed in the controls. More primordia were stimulated to growth with the addition of B_1 to the indolebutyric solution, and particularly so at 8 and 12 days. Root elongation, and to some degree the number formed, were inhibited in the naphthaleneacetic cultures. The phenylacetic cultures showed no significant differences in root length or number over the controls.

Growth of the young shoots was definitely inhibited, both in the indolebutyric cultures and in the mixture of B_1 and indolebutyric. The indoleacetic exhibited no inhibition to shoot growth when compared with the controls. There appeared to be no stimulation of shoot growth in any of the other cultures of the series.

By 8 days root hairs were formed on roots in most of the indole-compound cultures. In one tryptophane culture they were sparsely formed on one root 4.6 cm. long. In naphthaleneacetic most of the roots had formed them, even on those as short as 3.0 mm. Likewise in indolepropionic most of the roots had

formed them. In indoleacetic one root only, 2.4 cm. in length, had sparse root hairs. In the mixed cultures most of the roots had developed root hairs. None were formed in the controls, in the B_1, or in the phenylacetic at this period or by the end of 16 days.

In one of the stem segments included in one indolebutyric culture a callus formed at the cut surface at the proximal end. Apparently in cutting the segment some meristematic tissue of the bud node remained, for at 4 days a callus had developed, and by 8 days a small bud and a root 2.4 cm. long had formed. By 12 days there were three roots, and the bud still was dormant. Further experiments with this type of cut segment failed to show any such regeneration.

EXPERIMENT 2.—This experiment compares the responses of bud nodes to indoleacetic acid and l-tryptophane with greater replication of cultures than in experiment 1. By changing the solutions, a more or less constant concentration was maintained. The conditions for this experiment were much the same as for experiment 1. The type of material, manner of cutting, and selection were the same. Light conditions (quality, intensity, and duration) were the same. The compounds used were indoleacetic acid and l-tryptophane in concentrations of 1 p.p.m. The single bud-node segments were grown in 200 ml. of solution as in the previous experiment. In experiment 2, six bud nodes were placed in each glass, and no stem segments were used. Measurements were made and the solutions changed every 4 days. Each compound, and also the controls in tap water, were tested in replicates of six. The temperature range was the same as in experiment 1. This experiment ran August 7–20, 1941.

The observations (table 3) follow closely those made in experiment 1. The stability of aqueous solutions of indoleacetic acid has often been questioned. Stock solutions of the acid usually turn brownish, even in storage at low temperatures. This color change has not been observed in the other indole acids, including tryptophane, under the same storage conditions. It is also the only one of the indole acids studied to develop a characteristic odor upon standing at room temperature.

TABLE 3

GROWTH OF BUD NODES IN CULTURES OF INDOLEACETIC AND L-TRYPTOPHANE AT 1 P.P.M. AND IN CONTROLS. LENGTH VALUES (CM.) REPRESENT TOTALS FOR THIRTY-SIX BUD NODES

Days	No. roots emerged	Total length of roots	Ave. root length	Total length of shoots
	l-Tryptophane			
4	13	4.35	0.61	10.52
8	17	41.95	2.46	20.40
12	21	56.15	2.67	37.33
	Indoleacetic acid			
4	18	7.71	0.83	8.39
8	24	37.35	1.55	13.92
12	38	62.10	1.63	30.76
	Tap water			
4	1	0.05	0.05	10.81
8	2	1.15	0.57	20.72
12	17	24.54	1.44	31.45

It was not until about 12 days that extensive rooting of the controls occurred. During the period of the experiment only the first root appeared, and no root hairs were formed on any of these roots. In contrast to the controls, rooting was relatively more extensive in the indoleacetic

and tryptophane series at all times. The indoleacetic cultures at all three observation periods exceeded the other series in total root number. For the first 8 days these were all first roots. By the twelfth day, six of the bud nodes in indoleacetic had produced second roots. In the tryptophane cultures it was not until the twelfth day that a second root appeared, and this occurred in only one bud node.

In total root length, the indoleacetic cultures led all the series throughout the 12-day period, as indolepropionic did in experiment 1. In that experiment indoleacetic was very near to the second highest (indolebutyric) only at 16 days. Again, although indoleacetic led, it did not have the greatest average root length. With the exception of the first 4 days, the tryptophane cultures (as in experiment 1) had the highest average root length of all the series.

Growth of the buds was definitely inhibited in the indoleacetic cultures. This was particularly evident in the first 8 days of growth. At the twelfth day the values for indoleacetic and the controls were not so greatly different. In tryptophane for the first 8 days, the shoot growth was no greater than the controls. At 12 days, however, a small increase in shoot growth over the controls occurred in tryptophane.

The first roots to produce root hairs grew in the indoleacetic cultures; they were visible on the fourth day. The two roots observed were 0.8 and 9.5 cm. in length. None were observed in the tryptophane at 4, 8, or 12 days. At 8 days no new root hairs had formed in any of the series. At 12 days one additional root, 1.3 cm. in length, was formed in one of the indoleacetic series. It was densely covered with root hairs.

EXPERIMENT 3.—The responses of bud nodes to a series of relatively high concentrations of indoleacetic acid were tested. The same type of bud node was used as in previous experiments. They were grown, however, in 2-liter crocks in the full light of the greenhouse. In this experiment ten such nodes were grown in 1 liter of tap water with added indoleacetic acid. Duplicate cultures were not grown. The usual measurements were made at 6 and 13 days (table 4). The concentrations were 1, 5, 10, 15, 20, 25, and 30 p.p.m. The solution was not changed throughout the experiment. The cultures were grown under average greenhouse conditions through February 10–23, 1942. The temperature of the solutions frequently measured 22° C.

At 6 days the maximum activity occurred in the range from 5 to 10 p.p.m. The maximum numbers of roots (ten and eleven, respectively) also emerged at these concentrations. This was true at 13 days as well. In contrast to the indolebutyric and indolepropionic in previous experiments, the second root emerged from very few of the nodes in the entire series, and no third or fourth roots had appeared. The higher light intensity may be somewhat inhibitory, and a possible deterioration of this substance in water solution may have resulted in the generally poor responses. In agreement with experiment 1, the responses to indoleacetic at 1 p.p.m. were not appreciable. At both 6 and 13 days the data for the buds were somewhat variable, but apparently inhibition was relatively greater in the range 25–30 p.p.m. There was little significant stimulation of shoot growth by any of the lower concentrations when compared with the control. No root-hair formation was observed on the regular bud-node roots.

EXPERIMENT 4.—The responses of bud nodes to a series of indolepropionic acid at high dilutions were tested. Cultures

at a concentration of 5×10^{-3} gm./l. (5 p.p.m.), and of a series from 1×10^{-3} (1 p.p.m.) to 1×10^{-10} gm./l., were made up in tap water. The same type of bud node and the same manner of cutting and selection were used as in previous experiments. The series was more extensive, however, consisting of four glasses containing four bud nodes each for each of the concentrations and for the controls in tap water alone. The data (table 5) are based on measurements from sixteen bud nodes for each concentration. The usual measurements were made at 4-day intervals. The solutions were changed twice after the beginning of the experiment. The cultures were grown under fluorescent light of an intensity of 200 f.c., February 4–17, 1942.

The limit of significant growth response was at 10^{-4} (table 5), apparent from the total bud-length values both at 8 and 12 days, and the root values at 12. The low light intensity was of value in showing the shoot response at low concentrations, but it was too low for much root growth at 10^{-3}, and no root growth occurred in the controls.

The greatest response in root and shoot growth occurred at 5 p.p.m. Root emergence was most abundant, and root hairs were formed. In one glass, a root 0.25 cm. long had formed a band of very short root hairs. Shoot growth was nearly equal to that in the 10^{-4} series.

While the values for shoot growth in both 10^{-3} and 5 p.p.m. were nearly the same, the character of the growth was

TABLE 4

SHOOT AND ROOT GROWTH IN CONCENTRATIONS OF INDOLEACETIC ACID. LENGTHS (CM.) ARE TOTALS FOR TEN BUD NODES

Days	Concentration (p.p.m.)							
	0	1	5	10	15	20	25	30
	Total shoot length							
6	3.39	3.43	3.89	3.94	2.93	3.52	2.65	3.20
13	7.45	5.98	7.96	5.84	4.47	8.87	3.31	5.75
	Total root length							
6	3.92	3.72	14.58	15.42	4.10	4.70	2.00	8.34
13	14.95	13.15	52.80	40.02	15.00	27.50	2.80	18.90
	Average root length							
6	1.30	1.57	1.45	1.92	2.05	0.78	2.00	1.66
13	2.13	1.47	5.28	3.63	5.00	4.58	2.80	4.72
	No. of roots							
6	3	4	10	8	2	6	1	5
13	7	9	10	11	3	6	1	4

entirely different. At 10^{-4} the buds actually opened, and the shoot grew in an apparently normal fashion both by an elongation of the existent secondary axis and by the growth of the apical meristem. In the 5 p.p.m. cultures the shoot growth was distinctly different. In many cases the sole increase in the length of the bud was in the excessive growth of the meristematic region at the base of the secondary axis. This may or may not be accompanied by elongation of the secondary axis proper or by growth of the apical meristem. Sporadic growth of the entire shoot occurred in two bud nodes. One grew to a length of 2.4 cm. and another to 1.7 cm. Most of the buds, however, were 4–5 mm. in length.

In the cultures at 10^{-4} the increased growth was more uniform. Six of the shoots were over 8 mm. in length, while most of the rest were 3.5–6 mm. In comparison with data from the other experiments, this was considerably less growth; but when compared with the rest of the dilutions from 10^{-5} to 10^{-10}, the response at 10^{-4} would appear to be significant. Apparently the presence of indolepropionic acid in the cultures was related to growth of both roots and shoots at the low intensity employed. Without this substance (as in the controls), or in dilutions greater than 10^{-4}, no root or shoot growth occurred.

EXPERIMENT 5.—This experiment concerned the responses of bud nodes to indolebutyric acid at low light intensities. Cultures were run in duplicate with four single whorled bud nodes in each glass. Daylight fluorescent tubes were suspended over a series of shelves constructed in stair-step fashion to provide the light intensities. Two cultures in tap water, and two in indolebutyric at 1 p.p.m., were placed on the shelves under approximately 20, 40, 100, and 200 f.c., with an additional series in continuous darkness. Observations were made at 3, 15, and 21 days (table 6). The experiment ran January 14–February 4, 1942.

At the end of 15 days a small amount of shoot growth had occurred in the controls at 200 f.c., but no roots had appeared. On the other hand, in the indole-

TABLE 5

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF SIXTEEN NODES GROWN IN INDOLEPROPIONIC ACID AT LOW INTENSITY OF 200 F.C. LENGTHS IN CM.

CONCENTRATION (GM./L.)	GROWTH PERIOD								
	4 days			8 days			12 days		
	Total shoot	Total root	No. of roots	Total shoot	Total root	No. of roots	Total shoot	Total root	No. of roots
Control	4.81	.....	.....	4.47	.....	.....	4.70	.....	.....
1×10^{-10}	4.32	.....	.....	4.60	.....	.....	4.58	.....	.....
10^{-9}	4.54	.....	.....	4.67	.....	.....	4.81	.....	.....
10^{-8}	4.17	.....	.....	4.50	.....	.....	4.39	.....	.....
10^{-7}	4.34	.....	.....	4.89	.....	.....	4.45	.....	.....
10^{-6}	4.62	.....	.....	4.60	.....	.....	5.26	.....	.....
10^{-5}	4.58	.....	.....	4.67	.....	.....	4.76	.....	.....
10^{-4}	4.90	.....	.....	5.35	.....	.....	9.78	1.00	3
10^{-3}	4.67	.....	.....	4.75	1.39	2	5.51	1.04	2
5×10^{-3}	6.01	0.34	3	9.32	1.27	4	9.48	4.74	10

butyric cultures shoot growth was somewhat inhibited; while roots had emerged on over half the nodes. At 100 f.c. the controls showed no root emergence and little shoot growth, while in the indolebutyric, roots had emerged from over half the nodes and practically no shoot growth had occurred. At 40 f.c. there was no shoot or root growth in the controls, while in the indolebutyric small roots had emerged from half the nodes of shoot growth was still evident at all intensities and in the dark. Root growth occurred at all intensities, but not in the dark cultures. Only the first roots emerged by the end of 21 days in the indolebutyric cultures, and the maximum length of any root was 16 mm. at the highest intensity.

By the fifteenth day, the stumps of the nodes in the indolebutyric under the two lowest intensities, and in the dark as well,

TABLE 6

TOTAL ROOT AND SHOOT LENGTHS OF BUD NODES UNDER VARYING LIGHT INTENSITIES WITH AND WITHOUT INDOLEBUTYRIC ACID (1 P.P.M.). VALUES (MM.) ARE TOTALS FROM EIGHT NODES

Light intensity	Growth period											
	3 days				15 days				21 days			
	Shoot		Root		Shoot		Root		Shoot		Root	
	o	Ibc	o	Ibc	o	Ibc	o	Ibc	o	Ibc	o	Ibc
Darkness	22.2	26.4	.....	.....	22.0	21.9	.....	.....	23.6	22.3	.....	.....
20 f.c.	23.3	27.0	.....	.....	22.7	25.5	.....	2.2	24.2	18.7	.....	5.3
40 f.c.	25.1	25.9	.....	.....	23.5	22.2	.....	4.3	23.4	22.9	.....	1.6
100 f.c.	24.6	26.2	.....	.....	28.2	26.2	.....	20.3	31.4	25.3	.....	20.4
200 f.c.	24.5	26.0	.....	.....	30.5	27.3	.....	65.8	51.2	24.5	.....	67.2

but there was no shoot growth. At the lowest intensity, 20 f.c., the controls showed no root or shoot growth, while the indolebutyric had a few roots emerging with no shoot growth. The cultures in the dark showed no growth of roots or shoots.

At the end of 21 days the responses were little changed from those at 15 days. No roots had emerged from the control cultures at any light intensity or in the dark. Shoot growth in the controls was entirely limited to the two higher intensities, 200 and 100 f.c., although very few nodes at the latter intensity showed much bud growth. In the indolebutyric series, inhibition (or no visible response) were beginning to decompose. Axillary buds and leaves, however, appeared intact and green in color. In contrast, the controls at all intensities and in the dark appeared to have a greater number of nodes with sound axis tissue.

In some preliminary experiments with 25-cm. shoots in tap water alone and in 1 p.p.m. indolebutyric grown under conditions similar to the present one, considerable stimulation of both root and shoot growth occurred in the indolebutyric at the lowest intensity employed (20 f.c.). But at the higher intensities (46, 130, and 200 f.c.) inhibition in root elongation occurred in indolebutyric, and greater elongation occurred in the

controls. At this concentration indolebutyric produced little if any inhibition in shoot growth at these higher intensities.

From experiments 4 and 5, and also from the work on histidine, it would appear that photosynthate was produced at these lower intensities, but that in tap water alone little could be utilized for root and shoot growth. It would seem that the addition of indolebutyric, indolepropionic, and possibly histidine, in some manner aided the utilization of the photosynthate, and possibly stimulated the synthesis of total solids (**15**). From the work of MITCHELL *et al.* (**16, 17**) and EYSTER (**10**), it would appear that these substances might be related to the enzymatic hydrolysis of the stored starch. Other workers (**7**) have also shown the interrelationships between auxins and cellular respiration, salt absorption, and cell enlargement. CLARK *et al.* (**6**) found that intermittent treatment with phenylacetic, phenylpropionic, and indoleacetic produced increased reproduction in sterile *Lemna* cultures, with 0.1 p.p.m. giving the greatest stimulation. Indoleacetic was the least effective, however. From the present experiments it would seem that some naturally formed growth substance or substances associated with normal metabolism is not formed in sufficient amount for growth at these lower intensities. With the addition of certain substances, however, growth may be promoted for a time.[1]

TABLE 7

TOTAL SHOOT LENGTHS OF THREE BUD NODES AT 16 DAYS

Substance	Concentration (p.p.m.)	Tube no. 1 (mm.)	Tube no. 2 (mm.)
Glycine	5	6.9	6.2
Tryptophane	5	7.3	7.0
Histidine	5	30.5	26.5
Histidine	25	8.2	7.7
Control	0	7.6	7.8

EXPERIMENT 6.—This experiment was concerned with histidine. For comparison of effects, two other amino acids, tryptophane and glycine, were used. Histidine was used in the form of the salt, l-histidine monohydrochloride. The solutions, all at 5 p.p.m. (with a duplicate series of histidine at 25 p.p.m.), were grown in large test tubes in 60 ml. of tap water. The same type of bud node was used as in previous experiments. The cultures were grown in duplicate, with three bud nodes in each tube, in the full light of the greenhouse under conditions described for experiment 3. This ran February 12–28, 1942.

This small-scale experiment seems to indicate that histidine in some manner is related to bud growth, while the other amino acids are not, at least under the conditions of this experiment (table 7). Growth in the tubes was slow, and it was not until at 8 days that the buds were noticeable, when five out of six buds had opened. At 16 days the buds in 5 p.p.m. histidine had grown appreciably, apparently confirming the work of SOLTYS *et al.* (**23**). With intact shoots of *Elodea* they noted a growth of the axillary buds in solutions of l-histidine monohydrochloride. In the above, 25 p.p.m. histidine was inhibitory to shoot growth. In the present experiment no roots appeared in the culture period of 16 days. This lack of rooting was chiefly related to the low light in-

[1] MEYER *et al.* (Ecology **24**:393–399. 1943) have studied the apparent photosynthesis of certain submerged plants at various depths in Lake Erie. The compensation point for *Elodea canadensis* was attained at a depth of 8–10 m., with a light intensity range (1.03–0.27% of full sunlight) estimated from data of the present experiments to be equivalent to 99–27 f.c. of fluorescent light.

tensities prevailing during the growth period.

EXPERIMENT 7.—Cultures of l-histidine monohydrochloride at concentrations of 1, 2, 3, 4, 5, 8, 10, and 15 p.p.m. were grown at a light intensity of 350–450 f.c. Two series of cultures, one with short segments having one leaf whorl (lot 1) and one with longer segments having three whorls (lot 2), were grown at the concentrations named. The segments with one whorl had about eight leaves; those with three whorls had about sixteen. The data in table 8 are based on five segments for each culture. The experiment ran March 26–April 11, 1942.

TABLE 8

TOTAL ROOT AND SHOOT LENGTHS OF BUD NODES TREATED WITH HISTIDINE. LOT 1 HAD SHORT NODES WITH ONE WHORL OF LEAVES; LOT 2 HAD LONGER NODES WITH THREE WHORLS. VALUES (CM.) BASED ON SINGLE CULTURES OF FIVE NODES EACH

SOLUTION AND LOT	GROWTH PERIOD							
	4 days		8 days		12 days		16 days	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control								
1	1.37	0.04	1.96	1.00	4.47	7.20	4.64	12.60
2	1.77	0.05	3.70	3.37	6.90	26.40	8.50	35.65
1 p.p.m.								
1	1.51	0.05	2.76	1.10	4.53	16.05	4.99	19.65
2	1.50	0.04	3.29	2.87	6.52	27.17	8.18	33.63
2 p.p.m.								
1	1.57	.....	2.27	.....	4.73	.....	5.25	0.33
2	1.46	.....	1.77	.....	4.70	0.42	5.70	3.90
3 p.p.m.								
1	1.41	.....	2.27	.....	3.84	.....	4.55	0.08
2	1.35	0.05	1.74	0.20	2.72	3.70	4.37	4.90
4 p.p.m.								
1	1.42	.....	2.39	.....	5.00	.....	6.04	0.10
2	1.55	0.05	2.64	0.80	5.85	6.10	7.56	10.37
5 p.p.m.								
1	1.42	.....	1.83	.....	2.58	.....	2.99	.....
2	1.39	.....	2.07	.....	3.77	4.70	4.46	8.95
8 p.p.m.								
1	1.40	.....	1.52	.....	2.02	.....	2.53	.....
2	1.39	0.05	3.29	0.11	4.25	0.35	5.15	2.95
10 p.p.m.								
1	1.45	.....	2.16	.....	3.19	.....	3.57	0.04
2	1.51	0.02	2.32	.....	4.25	.....	5.55	.....
15 p.p.m.								
1	1.43	.....	1.56	.....	1.79	.....	2.00	.....
2	1.43	.....	1.57	.....	2.07	.....	2.32	.....

Table 8 shows the difference between the two lots with different-sized segments. With the larger ones (lot 2), root growth occurred in concentrations up to

8 p.p.m.; with the smaller segments (lot 1), little rooting occurred above 2 p.p.m. In the cultures of lot 1 there were practically no second roots formed, even at 1 p.p.m. In lot 2, second roots were formed only in the controls and in the 1 p.p.m. cultures. A few third roots were formed in the controls and in the 1 p.p.m. cultures of lot 2.

In lot 1, shoot inhibition was evident at 5 p.p.m., and also in lot 2 at the same concentration, with marked inhibition at 15 p.p.m. Inhibition of roots by concentrations higher than 1 p.p.m. may be in part an indirect one. All higher concentrations result in severe damage to the leaves of the segments. In the cultures having 1, 2, and sometimes 3 p.p.m., the leaves were bright green. At higher concentrations they became yellowish, and often the only green color was in the midveins. In older cultures these leaves became torn and decomposed. With leaf damage, sparse rooting generally followed.

EXPERIMENT 8.—This was designed to show possible correlations between light intensity and certain of the growth substances on the growth of excised bud nodes. In experiment 5 the cultures of indolebutyric at 1 p.p.m. maintained better growth of the segments at low intensities than the controls in tap water at such low intensities. In the present experiment, five bud nodes, each having one whorl of leaves, were grown in glass tumblers in 200 ml. of solution. The cultures were: duplicate cultures in tap water; duplicate cultures in histidine monohydrochloride at 5.0 p.p.m., and indolebutyric at 0.4 p.p.m.; and a single culture (five bud nodes) of histidine at 1.0 p.p.m. All solutions were made up with tap water.

The cultures were grown under different light conditions. One series of the cultures already described was placed in continuous darkness, another in daylight, and three were placed under three intensities of fluorescent light, obtained by the stair-step shelves as in experiment 5. A single fluorescent lighting unit, consisting of daylight tubes, was placed over the shelves. By this arrangement, three intensities of approximately 45, 200, and 400 f.c. were obtained. The cultures under fluorescent light were given 13 hours of light each day. The daylight series was grown under normal greenhouse conditions. The experiment ran April 22–May 8, 1942.

The cultures in the dark showed some root growth but practically no shoot growth (table 9). In many cases the bud scales opened, but no further growth occurred. The greatest number of roots (six at 16 days) and the greatest total root length (2.23 cm.) occurred in the dark series of histidine cultures (5 p.p.m.). While a number of roots emerged in the indolebutyric cultures, the roots remained very short.

In the cultures at the low light intensity of 45 f.c. there was marked difference in shoot growth. Both histidine at 5 p.p.m. and indolebutyric at 0.4 p.p.m. had a greater total shoot length than the controls at all three periods—8, 12, and 16 days. This effect of histidine is in agreement with a previous test. At low intensities, histidine and indolebutyric acid would appear to be associated with increased shoot growth. While the greatest number of roots (five) occurred in the indolebutyric at 16 days, there was little growth in length of these roots at this low intensity. Considerably greater total root length occurred in the 1 p.p.m. histidine than in the control.

At the higher intensities, 200 and 400 f.c., there was greater growth of both roots and shoots in most of the cultures.

TABLE 9

GROWTH RESPONSES OF CULTURES UNDER DIFFERENT LIGHT INTENSITIES. SHOOT AND ROOT LENGTHS (CM.) ARE TOTAL LENGTHS FROM TEN NODES (EXCEPT AS NOTED) IN EACH SERIES. NUMBER OF ROOTS REPRESENTS TOTALS FOR TEN NODES (EXCEPT AS NOTED)

CULTURE	CONCENTRATION (P.P.M.)	GROWTH PERIOD								
		8 days			12 days			16 days		
		Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots
		Darkness								
Control	0	3.20	0.25	2	3.39	0.46	3	3.21	1.24	3
Indolebutyric	0.4	3.02	0.35	4	3.39	0.44	4	3.34	0.70	5
Histidine	5.0	2.92	0.69	4	3.01	1.28	4	3.22	2.23	6
Control*	0	1.62	0.15	1	1.65	0.14	1	1.66	0.82	2
Histidine*	1.0	1.56	0	0	1.69	0	0	1.77	0	0
		45 foot candles								
Control	0	3.23	0.45	2	3.68	2.58	3	4.10	2.75	2
Indolebutyric	0.4	4.57	0.25	3	5.47	0.49	4	6.43	0.10	5
Histidine	5.0	5.27	0.45	2	6.03	2.40	2	6.50	4.24	3
Control*	0	1.53	0.05	1	1.72	0.08	1	1.99	0.05	1
Histidine*	1.0	1.94	1.05	2	2.21	4.95	2	2.44	5.75	2
		200 foot candles								
Control	0	7.54	3.97	4	9.60	5.15	3	11.40	3.85	4
Indolebutyric	0.4	6.41	6.37	4	8.57	15.08	6	10.01	19.25	6
Histidine	5.0	5.91	3.07	2	7.84	6.05	3	9.22	6.79	4
Control*	0	3.74	0.17	2	5.27	1.35	1	6.00	1.60	2
Histidine*	1.0	2.87	0.65	1	3.62	4.69	2	3.64	7.80	2
		400 foot candles								
Control	0	8.74	4.08	7	11.45	13.65	8	13.09	21.02	7
Indolebutyric	0.4	7.80	9.72	4	8.90	12.20	6	8.80	13.05	7
Histidine	5.0	6.92	0.25	1	8.83	1.89	2	9.78	2.78	3
Control*	0	4.87	0.42	4	6.40	7.05	4	7.04	8.32	3
Histidine*	1.0	4.37	1.51	5	5.65	12.75	5	6.10	16.00	4
		Daylight								
Control	0	6.38	0.30	3	8.92	0.80	5	11.32	2.02	7
Indolebutyric	0.4	5.58	5.30	4	7.87	1.89	8	10.33	8.20	10
Histidine	5.0	5.14	0	0	6.82	0.15	3	8.69	0.10	2
Control*	0	3.20	0	0	4.68	0.10	1	5.90	1.50	4
Histidine*	1.0	2.92	0.27	3	4.30	0.33	2	5.31	1.11	4

* Values for single control culture (five nodes only) and for single histidine culture at 1 p.p.m., respectively.

In the controls shoot growth was higher at these two intensities than at the lower ones and in daylight. Root growth in the controls reached a maximum at 16 days at 400 f.c. This was much greater than the value in the daylight controls. The total root growth in the indolebutyric was the highest at 16 days at 200 f.c. In the histidine at 5 p.p.m., inhibition of shoot growth occurred at both 200 and 400 f.c., while root growth was somewhat higher than the controls at 200 f.c. and decidedly inhibited at 400 f.c. In the 1 p.p.m. cultures of histidine there appeared to be greater root growth than in the controls at both 200 and 400 f.c.

In the daylight series, the total root and shoot lengths in almost every culture were considerably lower than those values at 400 f.c. While there may have been several other factors, such as greater temperature fluctuation, the very high light intensity was probably the chief.

It is interesting to note the differences in responses of this experiment and experiment 5. In this experiment some roots appeared on nodes in the control cultures in the dark and in the controls under the low intensities; in experiment 5 no roots appeared in the controls in the dark or under any of the low intensities. Possibly the time of year at which these nodes were cut was largely responsible for these differences. The nodes in experiment 5 were cut on January 14; those in experiment 8 were cut on April 22. Owing to cloudy weather, and the low temperature maintained in the house, there was little growth in the culture tanks during the winter months. BAUSOR (1) has pointed out that variations in the rooting of cuttings at different times of the year may be related to the reserve carbohydrate.

EXPERIMENT 9.—This was concerned with the responses of bud nodes to compounds at relatively high concentrations (5 p.p.m.). These bud nodes were cut differently. Instead of the one whorl of leaves on the bud segment, three successive whorls were left. The segments were longer, about 2.5–3.0 cm., thus having greater leaf and stem area than those previously used. A higher fluorescent light intensity, 420–440 f.c., was employed. The segments were grown five in each glass tumbler containing 200 ml. of solution. The water was obtained by filtering that from the tanks in which the stock cultures were growing. The temperatures during the daytime were generally 24°–28° C. The experiment ran February 19–28, 1942.

At higher light intensities, and with larger segments, growth of the roots and buds was more rapid. This greater growth may be related to the photosynthate (1). Table 10 shows the well-defined responses to certain of the compounds. The responses to treatment with the indole compounds were much the same as reported earlier. At 8 days indolepropionic acid was the highest of all in bud stimulation. Again this was due to a combination of the stimulation of the basal meristem of the secondary axis and some general growth of the latter. At 8 days the indolebutyric culture had the greatest percentage of roots per segment. Both the first and second roots had elongated in all five segments, while a third root emerged in one. Indoleacetic chiefly stimulated growth of the first root; in only two segments had the second root emerged. The average root length was great here also. The average was quite low in indolepropionic and indolebutyric but high again in tryptophane. The greater root elongation occurring in the tryptophane may be related to its role as an amino acid. This is apparent when glycine (which has the amino group but

no indole configuration) produces about the same responses. Growth in histidine was not pronounced, but shoot growth was somewhat greater than the controls.

Phenylacetic and thiophenyl acetamide did not produce definite gross responses in any of the cultures. Naphthaleneacetic is definitely inhibitory and approaches toxicity at 5 p.p.m. The three vitamins appear to stimulate rooting to a limited degree, especially ascorbic acid.

EXPERIMENT 10.—This was designed to study the responses of excised segments to a series of amino acids and some other growth substances. The segments of three whorls were grown under conditions similar to those of preceding experiments, under fluorescent lights at an average intensity of 400 f.c. Duplicate cultures of five segments each were grown for a period of 20 days. The substances used were histidine monohydrochloride, l-tryptophane, glycine, urea, asparagine, thiamin hydrochloride, nicotinic acid, ascorbic acid, and calcium pantothenate. The solutions were made up with tap water at a concentration of 5 p.p.m., and the original solutions were replaced with fresh ones at 17 days. The experiment ran March 31–April 20, 1942.

Ascorbic acid and calcium pantothenate were associated with the highest rooting activity at 12, 16, and 20 days. Plants in these, together with B_1 and tryptophane, cultures were consistently high in rooting activity during the 20 days. Responses in cultures of glycine, nicotinic acid, and asparagine, while not so marked as in the cultures previously listed, were still higher in root growth than the controls. Histidine was almost completely inhibitory to root growth (table 11). Although ascorbic acid (vitamin C) has been reported to oxidize readily, this substance or its changed form appeared to stimulate root growth.

In the number of roots formed by the end of the 20-day period, pantothenate and tryptophane led (twenty and twenty-one, respectively), while the controls had a total of ten roots. The lowest numbers were in histidine and asparagine—three and seven, respectively. The cultures containing ascorbic acid, B_1, and urea formed more roots than the controls. Not more than one primordium per node was stimulated to growth in the histidine cultures, and then only a few roots were formed. At the end of 20 days, second roots only were formed in the B_1; glycine, asparagine, and control series. The largest number (three) of third roots was in the tryptophane cultures, while two segments had third roots in each of the cultures of ascorbic, nicotinic, pantothenate, and urea.

The growth differences of the shoots were not so striking. Definite inhibition of the shoot occurred with histidine and to a lesser extent with tryptophane. No very great increase of shoot growth could

TABLE 10

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF BUD NODES GROWN AT 5 P.P.M. LENGTH VALUES (CM.) ARE TOTAL LENGTHS OF SHOOTS AND ROOTS OF FIVE BUD NODES

CULTURE	GROWTH PERIOD					
	4 days			8 days		
	Total shoot	Total root	No. of roots	Total shoot	Total root	No. of roots
Indoleacetic	1.55	0.58	4	3.80	18.80	7
Indolepropionic	2.15	0.68	4	6.05	4.75	8
Indolebutyric	1.98	0.73	4	3.72	9.16	11
l-Tryptophane	1.67	0.78	5	4.80	20.65	7
Glycine	2.17	0.64	5	4.50	21.26	7
Histidine	2.00	0.43	5	4.95	12.47	5
Phenylacetic	1.46	0.10	2	4.23	7.15	4
Thiophenyl acetamide	1.47	0.26	3	3.45	3.83	4
Naphthaleneacetic	1.82	0.30	2	2.77	0.30	2
Nicotinic acid	1.52	0.09	2	3.63	12.05	4
Ascorbic acid	1.85	0.15	1	5.15	12.75	7
Vitamin B_1	1.71	0.25	5	4.25	12.70	5
Control	1.65	0.40	4	3.58	9.25	6

be correlated with any of the compounds at 5 p.p.m.

Inhibitory responses of shoot growth in tryptophane are in contrast to the general shoot stimulation noted in indolepropionic cultures in other experiments. The total shoot length of the tryptophane cultures (10.99 cm.) at 20 days was considerably lower than the controls (16.86 cm.). In other experiments, shoots in indolepropionic have centration of 100 p.p.m. This increased growth in glycine, and in urea also, may be due in part to utilization of the amino nitrogen present in these compounds. WHITE (**25**) has found that glycine alone could substitute for all the amino acids formerly required in the nutrient used for certain root-tip cultures.

EXPERIMENT 11.—A series of cultures with concentrations of alpha naphthalene acetamide at 0.5, 2.5, 5.0, 7.5, and

TABLE 11

TOTAL ROOT AND SHOOT LENGTHS OF NODES GROWN IN CULTURES OF DIFFERENT COMPOUNDS AT 5 P.P.M. VALUES (CM.) ARE TOTALS FROM TEN NODES

SOLUTION	GROWTH PERIOD									
	4 days		8 days		12 days		16 days		20 days	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Urea	2.78	.....	6.57	1.32	11.83	16.33	15.25	52.92	16.70	59.55
Vitamin B$_1$	3.01	0.29	6.58	6.16	11.40	25.97	15.45	49.88	17.15	72.50
Histidine	3.00	0.03	4.02	0.17	5.60	0.35	7.84	2.20	9.35	3.18
Ascorbic	2.99	0.18	6.52	9.75	11.03	40.69	15.06	63.62	17.55	76.39
Nicotinic	2.75	0.05	3.79	1.19	6.89	7.88	6.55	13.50	14.34	29.33
Asparagine	2.74	.....	4.71	0.70	8.17	6.22	12.75	17.02	13.79	21.45
Glycine	3.08	0.10	4.93	0.44	9.00	6.65	12.85	23.21	16.15	33.28
Calcium pantothenate	2.99	0.09	6.16	3.88	10.61	36.29	14.35	62.40	15.76	73.20
Tryptophane	2.93	0.86	3.67	11.14	4.36	31.50	6.53	52.67	10.99	62.53
Control	3.03	.....	4.91	.....	9.00	.....	14.07	9.84	16.86	16.90

generally been much greater than the controls. This differential response would not seem to be correlated with root growth, since root stimulation is much the same in cultures of these two compounds. This difference of response in shoot elongation was also evident in experiment 13.

In experiments with a greater range of concentrations of glycine—not reported here—the greatest total root lengths and the greatest number developed in 1 p.p.m. Shoot growth could not be correlated very well with the various concentrations. No inhibition in shoot growth occurred, however, even at the high con- 10.0 p.p.m. were grown under fluorescent lights at an average intensity of 400 f.c. Duplicate cultures, each with five bud nodes of three leaf whorls, were used. The experiment ran April 2–22, 1942.

Roots appeared rapidly in the 5 p.p.m. cultures, for at 3 days roots had emerged from nine of the ten nodes; while only two nodes in the controls had rooted (table 12). At 12, 15, and 20 days the greatest total root length, and the greatest number of roots, occurred at 2.5 p.p.m. At 15 and 20 days both the root lengths and the number emerging were inhibited at 10 p.p.m. When the five longest roots in each culture were aver-

aged, the greatest average root length was 6.7 cm. for the 12- and 15-day periods at 2.5 p.p.m. (6.2 cm. at 12 days in 0.5 p.p.m. cultures), while the controls averaged only 3.9 cm. at 12 days and 4.0 cm. at 15 days. At 20 days the averages were much as just described.

In other experiments with lower concentrations of naphthalene acetamide, not reported here, the roots attained great lengths. In the controls the roots began to die when reaching lengths of 4 or 5 cm., while in cultures at 0.2, 0.3, 0.4, and 0.5 p.p.m. roots attained lengths of 8, 9, and 10 cm. The longest root ever measured in cultures grown in glass tumblers was 11.8 cm. at 17 days in the 0.2 p.p.m. In these experiments root hairs were observed on roots at 3.0 p.p.m.

In the present experiment fourth roots appeared at 15 days in the 0.5, 2.5, and 5 p.p.m. cultures. At this period the 2.5 p.p.m. cultures were the only ones in which all nodes had third and fourth roots. No third or fourth roots had yet appeared in the controls, eight nodes having rooted, with only three having the second root. At 20 days fifth roots appeared in the 0.5, 2.5, and 5 p.p.m. cultures; no fourth or fifth roots had appeared in the controls or at 10 p.p.m.

Root hairs were observed on some of the distal portions of the longer roots in the 0.5 p.p.m. at 15 days. Root hairs were not observed in the higher concentrations. There was little correlation of shoot growth with the various concentrations. At 10 p.p.m., however, shoot growth appeared to be definitely inhibited.

There are striking differences in the responses to naphthaleneacetic acid and to naphthalene acetamide at the same concentrations. Root-hair formation is very profuse with naphthaleneacetic at 1.0 p.p.m., and this concentration is highly inhibitory to root elongation, since most roots rarely attain lengths greater than 1–2 cm. This concentration is also inhibitory to shoot growth. In naphthalene acetamide, root-hair formation is sparse and has been observed at concentrations of 0.5 and 3.0 p.p.m., while inhibition in root elongation is not very marked, even at concentrations of 10 p.p.m.

TABLE 12

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF TEN NODES IN ALPHA NAPHTHALENE ACETAMIDE. LENGTHS (CM.) ARE TOTALS FOR TEN NODES

Concentration (p.p.m.)	Growth period														
	3 days			8 days			12 days			15 days			20 days		
	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots
0.....	3.25	0.15	2	6.52	1.85	4	11.70	11.54	7	16.99	29.59	11	20.65	39.05	12
0.5.....	3.17	0.38	5	7.41	12.35	9	12.36	51.32	10	16.75	88.97	27	17.60	80.95	31
2.5.....	3.32	0.90	6	5.12	25.43	13	7.04	72.56	23	8.75	103.00	33	9.02	106.66	40
5.0.....	3.50	1.35	9	4.94	33.28	13	7.49	48.92	19	11.91	69.10	31	11.05	73.18	33
7.5.....	3.56	0.69	6	4.32	12.49	10	5.67	31.98	13	7.05	42.04	21	7.90	43.50	26
10.0.....	2.95	0.66	7	3.68	13.22	10	4.01	20.02	12	4.64	22.75	13	4.67	18.45	12

EXPERIMENT 12.—In experiment 1 it was observed that, in a mixture of vita-

min B_1 and indolebutyric acid (each at 1 p.p.m.), root emergence was somewhat greater than in the single cultures of indolebutyric. This mixture was tried again, using each at 5 p.p.m. Four three-whorled segments were used in each glass, with each series containing four glasses. Filtered water from the culture tank was used. The cultures were grown under daylight fluorescent lights of about 400 f.c. The experiment ran March 13–April 1, 1942.

At 12 days there was marked inhibition of shoot growth in the indolebutyric cultures, while the difference between the mixture and the control was not so great (table 13). There was about a 25% greater total root length in the B_1 cultures at 12 days than in the controls. The indolebutyric and the mixture were about the same—only about one-third as great as the controls in total root lengths. A comparison of the five greatest root lengths in each of the series showed the longest in the B_1 cultures. At 16 days there appeared to be a little less inhibition of shoot growth in the mixture than in the single indolebutyric cultures.

At 12 days there were about four times as many nodes having third roots in the mixture as in the controls. At 16 days fourth roots were formed in the indolebutyric and in the mixture, while in the controls and in the B_1 cultures the maximum number per node was three. A fifth root was produced on one node in the mixture at this growth period. Except for a greater stimulation of third and fourth roots, the greater total number of primordia stimulated in the mixture at 5 p.p.m. was less evident than in the mixture at 1 p.p.m. in experiment 1.

EXPERIMENT 13.—This was designed to study the elongation rates of apical segments in various substances in the light and in the dark. Apical segments 4 cm. in length were cut from shoots of *Elodea* growing in the culture tanks. Four segments were placed in each glass tumbler, and two series, one in light and one in continuous darkness, were run simultaneously. There were no duplicate cultures in either series. The segments were grown in a nutrient solution consisting of 0.0045 mol. $MgSO_4$, 0.0045 mol. KH_2PO_4, 0.0060 mol. $Ca(NO_3)_2$, together with the minor elements diluted 1:20. The various substances were then added at a concentration of 5 p.p.m. Those used were indoleacetic, indolepropionic, indolebutyric, naphthaleneacetic, alpha naphthalene acetamide, l-tryptophane, vitamin B_1, ascorbic acid, calcium pantothenate, and l-histidine monohydrochloride. The cultures in the light were grown under fluorescent light for 13 hours each day at about 400 f.c. The other series was grown in continuous darkness, except for the very short time used in measuring. Measurements of the segments in the dark were made with the aid of a faint green light approximately every 20 hours. The temperatures of the two series did not differ greatly. For example, on May 6 at 7:30 P.M. the water temperature was 23.8° C. in the dark series and 22.0° C. in the light series. The experiment ran May 7–13, 1942.

Growth rates were calculated on the basis of the total growth increment of the four segments in each culture for each of the approximately 20-hour periods (table 14). The control culture referred to is the one at the 1:20 dilution, since for comparison another control in 1:1 nutrient was also used. Preliminary experiments with apical segments had shown that if the leaves were stripped back about 10–15 mm. from the growing point, there was some elongation in tryptophane at 5 p.p.m. in the dark. No expansion of the tiny leaves or growth at

the apical meristem appeared to take place. Increase in length of the segments was apparently due entirely to elongation of the internodes, principally those in the apical third of the segment.

The segments elongated somewhat during the first 21 hours in all cultures of both series. The highest growth rate for this first 21 hours, 0.30 cm./hr., was in the indoleacetic dark culture, while the rate was 0.13 cm./hr. in the dark control. The second highest rate for this period, 0.29 cm./hr., was in the naphthaleneacetic dark culture. The greatest decrease in growth rate in the second period (41 hours) was in both the dark and light series in the indoleacetic cultures. This possibly indicates a deterioration of the compound in water solution, since the cultures of the other indole compounds generally showed a higher growth rate at the end of the 41-hour period.

The naphthalene acetamide dark culture was the only one in the entire series which had a higher growth rate at the end of 41 hours than at the end of 21 hours. The highest growth rate maintained in the later periods was in the naphthaleneacetic dark culture, with 0.14 cm./hr. at the end of 102 hours. The rate in this culture at 129 hours was also the highest for any of the cultures.

Practically no stimulation of the segments occurred in the tryptophane cultures, either in the dark or the light series. In fact, tryptophane appeared to inhibit elongation in the light series. The ascorbic-acid, pantothenate, and histidine cultures all had greater growth rates than the controls in the dark; while in the light there was no appreciable stimulation. The growth rate in the histidine dark culture remained relatively high during the first three growth periods (up

TABLE 13

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF BUD NODES GROWN IN 5 P.P.M. OF INDOLEBUTYRIC ACID AND 5 P.P.M. OF VITAMIN B_1, AND IN MIXTURE OF THESE TWO SUBSTANCES EACH AT 5 P.P.M. LENGTH VALUES (CM.) ARE TOTAL LENGTHS OF SHOOTS AND ROOTS OF EIGHT NODES. VALUES FOR TWO LOTS (EACH OF TWO CULTURES) INCLUDED

Culture and lot	Growth period											
	4 days			8 days			12 days			16 days		
	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots
Control												
1	2.81	0.33	5	6.25	9.40	7	10.93	34.64	16	15.65	81.05	22
2	2.95	0.46	6	6.21	12.80	9	11.10	38.88	16	15.85	86.15	22
Vitamin B_1												
1	3.01	0.47	5	7.40	15.44	7	12.35	49.97	17	17.15	112.45	22
2	2.70	0.40	4	6.55	13.25	8	10.55	50.33	14	13.95	93.09	21
Indolebutyric												
1	3.08	1.05	8	4.04	6.64	13	6.91	13.90	17	10.60	21.60	22
2	2.76	0.92	5	3.88	4.30	11	6.35	13.15	17	11.05	22.50	22
Mixture												
1	2.83	0.85	7	5.73	6.32	13	8.55	13.75	20	12.90	18.80	22
2	3.15	1.31	7	4.96	5.40	12	10.80	12.82	20	18.20	23.05	26

TABLE 14

TOTAL LENGTHS, GROWTH INCREMENT, AND MEAN HOURLY GROWTH RATE IN CM./HR., FOR TOTAL OF FOUR SEGMENTS IN EACH CULTURE OF LIGHT AND DARK SERIES FOR EACH GROWTH PERIOD. MEASUREMENTS (CM.) ARE TOTAL VALUES FOR THE FOUR SEGMENTS IN EACH CULTURE

SOLUTION		HOURS											
		21		41		62		80		102		129	
		Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Indoleacetic	T*	19.80	22.22	21.72	26.35	22.75	26.90	23.23	27.39	23.85	27.40	24.87	27.95
	I	3.80	6.22	1.92	4.13	1.03	0.55	0.48	0.49	0.62	0.01	1.02	0.55
	R	0.18	0.30	0.10	0.21	0.05	0.03	0.03	0.03	0.03	0.00	0.04	0.02
Indolepro-pionic	T	19.29	21.98	20.50	27.60	22.20	32.45	23.30	35.60	24.80	37.50	26.29	38.98
	I	3.29	5.98	1.21	5.62	1.70	4.85	1.10	3.15	1.50	1.90	1.49	1.48
	R	0.16	0.28	0.06	0.28	0.08	0.23	0.05	0.15	0.07	0.09	0.06	0.05
Indole-butyric	T	18.96	21.20	19.91	25.21	20.40	29.61	21.15	32.07	22.20	33.90	23.10	35.18
	I	2.96	5.20	0.95	4.01	0.53	4.40	0.71	2.46	1.05	1.83	0.90	1.28
	R	0.14	0.25	0.05	0.20	0.03	0.21	0.04	0.14	0.05	0.08	0.03	0.05
Naphtha-leneacetic	T	19.27	22.03	21.67	27.34	24.82	32.05	26.95	35.32	28.35	38.40	29.55	40.00
	I	3.27	6.03	2.40	5.31	3.15	4.71	2.13	3.27	1.40	3.08	1.20	1.60
	R	0.16	0.29	0.12	0.27	0.15	0.22	0.12	0.18	0.06	0.14	0.04	0.07
Naphtha-lene ace-tamide	T	18.30	21.75	20.10	27.65	21.45	31.40	22.38	33.05	23.65	34.30	26.00	35.40
	I	2.30	5.75	1.86	5.90	1.29	3.75	0.93	1.65	1.27	1.25	2.35	1.10
	R	0.11	0.27	0.09	0.30	0.06	0.18	0.05	0.09	0.06	0.06	0.09	0.04
Vitamin B_1	T	16.57	19.18	16.75	21.35	17.11	23.07	17.23	24.00	17.72	24.70	18.13	24.21
	I	0.57	3.18	0.18	2.17	0.36	1.72	0.12	0.93	0.49	0.70	0.41	0.00
	R	0.03	0.16	0.01	0.11	0.02	0.08	0.01	0.05	0.02	0.03	0.02	0.00
Ascorbic	T	16.60	20.03	16.74	23.27	17.27	25.05	17.30	27.60	17.97	26.45	18.58	26.92
	I	0.60	4.03	0.10	3.24	0.53	1.78	0.03	2.55	0.67	0.00	0.61	0.00
	R	0.03	0.19	0.04	0.16	0.03	0.08	0.00	0.14	0.03	0.00	0.02	0.00
l-Histidine	T	16.80	19.43	17.00	22.19	16.98	22.55	17.20	23.08	17.74	23.80	18.34	24.12
	I	0.80	3.43	0.20	2.76	0.00	0.36	0.22	0.53	0.54	0.72	0.60	0.32
	R	0.38	0.16	0.01	0.14	0.00	0.17	0.01	0.03	0.02	0.03	0.02	0.01
l-Trypto-phane	T	17.31	19.60	17.77	22.55	18.07	23.70	18.11	24.75	18.58	25.45	19.19	25.75
	I	1.31	3.60	0.46	2.95	0.30	1.15	0.04	1.05	0.47	0.70	0.61	0.30
	R	0.06	0.02	0.02	0.15	0.01	0.05	0.00	0.05	0.02	0.03	0.02	0.01
Calcium pan-tothenate	T	16.99	18.25	17.32	19.24	17.88	19.40	18.11	19.50	18.67	19.75	19.45	19.95
	I	0.99	2.25	0.33	0.99	0.56	0.16	0.23	0.10	0.56	0.25	0.78	0.20
	R	0.05	0.11	0.02	0.05	0.03	0.01	0.01	0.01	0.03	0.01	0.03	0.01
Nutrient (1:1)	T	16.57	17.48	17.05	19.70	17.76	20.70	18.27	21.34	18.83	22.05	19.81	22.90
	I	0.57	1.48	0.48	2.22	0.71	1.00	0.51	0.64	0.56	0.71	0.98	0.85
	R	0.03	0.07	0.02	0.11	0.03	0.05	0.03	0.04	0.03	0.03	0.04	0.03
Nutrient (1:20)	T	17.28	18.70	17.72	20.15	17.24	20.75	18.75	20.72	19.38	22.30	22.72	21.17
	I	1.28	2.70	0.44	1.45	0.00	0.60	1.51	0.00	0.63	1.58	3.34	0.00
	R	0.06	0.13	0.02	0.07	0.00	0.03	0.08	0.00	0.03	0.07	0.12	0.00

* T, total length of four segments in each culture; I, total growth increment of the four segments; R, mean hourly growth rate in cm./hr. for total growth increment of the four segments for each period.

to 62 hours); while inhibition definitely occurred in the light culture throughout the entire 129 hours.

In all but one of the light cultures the growth rate was lower up to the end of 41 hours than the rate in the dark series. The highest growth rate in the light series occurred in the first 21 hours in the indoleacetic, 0.18 cm./hr.; in the control it was 0.06 cm./hr. There was prolonged growth rate in the naphthaleneacetic light culture. This rate at the end of 62 hours, 0.15 cm./hr., was the highest of all in the light series at that period. The least inhibition of the growth rate by light in any of the cultures was in the indoleacetic culture in the first 21 hours. A clue to the relationships of auxins and light is to be found in the work of EYSTER (10).

Definite inhibition in growth rate occurred in the ascorbic, B_1, and histidine light cultures: all were lower than the control. Calcium pantothenate and tryptophane were inhibitory to growth for all periods after the first 21 hours.

In the matter of the greatest elongation in the dark, one segment in the indolepropionic solution measured 10.9 cm., the greatest in the entire series. This was a 36% increase over the original 4-cm. segment in the growth period of 129 hours. The greatest total increment for the four segments in any one culture for the total growth period of 129 hours was in both the dark and light naphthaleneacetic cultures. The next highest in total increment were the dark and light cultures in indolepropionic.

The presence of the amino group in the l-tryptophane molecule appears radically to alter the properties of this substance when the responses of indolepropionic are considered. In the previous experiments with indolepropionic, root elongation was somewhat inhibited, but at the same time elongation of the secondary axis was greatly increased. Tryptophane, however, does not stimulate axis elongation. The theory that growth responses resulting from the application of tryptophane are due to indoleacetic acid, the latter originating through chemical change of tryptophane, has been questioned by KRAUS (13). The experiments with apical segments indicated that tryptophane did not show characteristic indoleacetic responses. It must be recognized, however, that tryptophane, as well as the other substances used in these experiments, may undergo a change in non-sterile aqueous solutions.

ROOT-HAIR FORMATION

The rooting reactions of species of this genus have been noted (22, 26, 8). Ordinarily, the free-growing roots of *Elodea* do not form root hairs in water. Generally such hairs are produced (*a*) in darkness; (*b*) upon penetration of the root into soil; (*c*) by treatment with ethylene gas; and (*d*) when the roots are in contact with a medium such as quartz sand.

In some preliminary investigations upon the effects of indolebutyric acid on 25-cm. shoots of *Elodea*, it was noted that root hairs were formed on most of the roots in concentrations of 1 p.p.m. This occurred in cultures grown in the full light of the greenhouse as well as in those grown under fluorescent lights of 200 f.c. With the use of excised bud nodes in the experiments described here, it has been observed that—in addition to indolebutyric acid—indolepropionic acid, naphthaleneacetic acid, alpha naphthalene acetamide (and to a lesser degree indoleacetic acid and l-tryptophane) stimulated root-hair formation. Of all the different types of compounds used in these experiments, only those

with the indole or naphthalene ring system—and acid side chains of two up to four carbons—were associated with root-hair formation.

CORMACK (8) states that certain short cells are cut off early in the development of the epidermis. These have been termed piliferous cells, since these are the only ones which produce hairs. In the case of treatment with indolebutyric acid at least, these special cells are the only ones to develop root hairs. On roots growing in water, the piliferous cells soon lose their identity and in the mature region of the root have the same shape as the rest of the elongated epidermal cells. In roots penetrating soil, the piliferous cells develop hairs and then these cells elongate as do the rest. When grown in 5 p.p.m. indolebutyric acid, the roots were rather rigid. Root hairs were formed abundantly, and elongation was inhibited to some degree. Piliferous cells, all with root hairs, still retained their characteristic cell shape, even in the mature region of the root. There was considerable thickening of the walls of the cells in the outer regions of the cortex. In some, the smaller intercellular spaces had been almost obliterated by the extensive thickening of the bounding walls. The stelar region seemed not to have undergone any thickening.

In root hairs formed in 1 p.p.m. indolepropionic acid, cells were often forked terminally. This was more evident when developed in 5 p.p.m. In the latter case the hair cells were not only frequently forked, but often many buds and branches appeared. Often the cells were club-shaped, with the terminal portion considerably swollen.

CORMACK (8) found that ethylene gas stimulated root-hair formation in *Elodea*. Although he attributed this to an inhibition in cuticle formation, BORGSTRÖM (2) —on the basis of his own work and data from the work of CROCKER, HITCHCOCK, and ZIMMERMAN (9) and others—has proposed that root-hair formation is an auxin response. He states that ethylene produces a transverse movement of auxin from the phloem to the epidermis.

In the light, root hairs do not develop on free-growing roots, even under the low intensities used in these experiments. Large segments placed in continuous darkness in tap water did form roots with hairs. This inhibition of root-hair formation in the light is probably related to auxin utilization. The somewhat inhibitory action of light on the elongation of apical segments is evident in experiment 13. EYSTER (10) has suggested that light accelerates the rapidity with which enzymes are bound to a colloidal carrier, consequently making them less free to act, and that growth substances merely aid in releasing the enzyme from the colloid. But just how the auxins are associated with the extensibility of the cell wall has remained until recently largely unknown. COMMONER, FOGEL, and MULLER (7) have experimented with the effect of auxin on water absorption by potato tuber tissue. Their results indicate that an auxin system regulates salt absorption processes in plant cells and through this effect influences water relations and cell enlargement.

Still another interpretation of cell elongation has been proposed by BURSTRÖM (3). In studies of the effects of indoleacetic on wheat roots, he differentiates two actions. The first is an accelerated rate of growth with increased wall plasticity before the start of elongation, and the second is an inhibition phase. "The action of indoleacetic is supposed to be a similar loosening of molecular joints during both phases of the elongation; during the first phase it increases

the swelling of the pectic (intermicellary) substances, during the second phase it prevents the deposition of cellulose micelles." He notes that roots recover from the inhibition, and may become adapted to even higher concentrations of indoleacetic.

The regulatory roles of growth substances just described may have been in large part responsible for the structural abnormalities of root hairs developed in indolepropionic solutions. While the hair cells developed no great lengths, their forked and greatly swollen apices indicated abnormal water relations, induced and greatly magnified by treatment with a high concentration of indolepropionic acid.

Some of the experiments described here were exploratory and the data obtained are presented despite the fact that they may not be wholly conclusive.

Summary

1. Since *Elodea* is an aquatic, excised bud-node and apical segments were readily grown in aqueous solutions of some sixteen growth substances and related compounds. The principal gross responses noted were axillary bud development, shoot elongation, the number of roots emerging, time and order of their appearance, their lengths, and root-hair development.

2. The injury of excision provided the initial stimulus for renewed growth in the bud nodes. Although some minor modifications were noted, where damage through treatment had not occurred, the general pattern of vegetative reproduction remained relatively unaltered in both control and test solutions. Excised bud nodes, in tap water alone, and cultured under suitable growing conditions, developed into complete new plants.

3. Nearly all the cultures were grown in 250-ml. glass tumblers under daylight fluorescent light with a daily light period of 13 hours. Experiments with different intensities of light showed that a reasonably good growth was maintained at 400 f.c.

4. In this species four root primordia are formed at each bud node very early in the ontogeny of the primary axis. There are two latent primordia in the primary axis in the region of divergence of the secondary axis, and two in the base of the secondary axis near the point of divergence from the primary axis. Even in rooted shoots as long as 1 m. these primordia remain latent. Upon excision of the bud node the two primary primordia emerge, and later the two secondary primordia may emerge. In control cultures in tap water these secondary primordia rarely appear, at least in the culture period of about 20 days. When these nodes are grown in solutions of certain indole or naphthalene compounds, stimulation of all four primordia may occur, and occasionally fifth roots may emerge.

5. For total root lengths at 16 days (experiment 1, concentration 1 p.p.m.) the decreasing magnitude of response was: indolepropionic, indolebutyric, indoleacetic, l-tryptophane, phenylacetic, control, B_1, and naphthaleneacetic. Average individual root length was: l-tryptophane, indolepropionic, control, phenylacetic, indoleacetic, B_1, indolebutyric, and naphthaleneacetic. For total number of roots the order was: indolebutyric, indolepropionic, indoleacetic, naphthaleneacetic (l-tryptophane, phenylacetic, and B_1 all similar), and control.

6. For total elongation of apical segments in the dark series at 129 hours (experiment 13, concentration 5 p.p.m.) the decreasing magnitude of response was: naphthaleneacetic, indolepropionic,

naphthalene acetamide, indolebutyric, indoleacetic, ascorbic, l-tryptophane (B_1 and histidine similar), and control. In the light series the order was the same through acetamide, and then the order was indoleacetic, indolebutyric, and control. The remainder of the substances had some inhibitory effect in the light. The highest growth rate measured was 0.30 cm./hr. (control, 0.13 cm./hr.) in the indoleacetic dark series at 21 hours.

7. Root hairs were rarely formed on the free-growing roots of excised bud nodes, larger segments, or shoots, growing under adequate light intensities. Root-hair formation did occur upon treatment with certain types of compounds. Of all the different types used, only those compounds with the indole or naphthalene ring system (and containing acid side chains) were associated with root-hair formation. In cultures in indolepropionic at 5 p.p.m., structural abnormalities developed in the terminal portions of the hair cells—possibly related to the abnormal water relations induced and greatly magnified by this high concentration. In view of the specificity of the response to certain types of compounds, this root-hair test of *Elodea* would appear to be a useful method of establishing a diagnostic character for the classification and characterization of certain of the growth-regulating substances.

8. The degree of inhibition and stimulation induced by many of the growth substances (aside from concentration) was a function of the light intensity, and to some extent the size of the segment employed. Increased growth of roots and shoots was associated with indolebutyric, indolepropionic, and histidine at low light intensities, where in control cultures little or no growth occurred. The association of growth-regulating substances with the synthesis of total solids, with starch hydrolysis, cellular respiration, salt absorption, and water regulation was considered in relation to cell elongation and to other growth processes.

The writer expresses his appreciation for the assistance given by Dr. C. A. SHULL and other members of the Department of Botany of the University of Chicago during the progress of this work.

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EFFECTS OF OSMOTIC CONCENTRATION OF SUBSTRATE ON THE ENTRY OF WATER INTO CORN ROOTS

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Introduction

In a previous paper a potometric device for measuring the entry of water into roots was described, and quantitative data for corn and *Citrus* were reported (5). The studies with corn indicated that the rate of entry increases from a zone proximal to the root cap to a maximum at a point approximately 10 cm. from the root tip and that, in most cases, the rate decreases above this level. The studies reported here were initiated to determine the effect of the osmotic concentration of the substrate on entry of water, since this relationship has a bearing on problems encountered in irrigation agriculture where saline conditions exist. Supplementary experiments were undertaken to investigate the effect of removal of the shoot on entry of water into the roots.

Procedure

The experimental room was maintained at a temperature of 72° F. ±1, with a relative humidity of 40% ±5. Overhead illumination was supplied by Westinghouse white fluorescent tubes. The room was equipped with six aquaria, and six plants were used in each experimental run (fig. 1).

Mexican June corn was used as the test plant. The seeds were germinated in sphagnum and the seedlings transferred to aerated water cultures containing a complete nutrient solution. The young plants were grown in the greenhouse until they were 10–12 inches tall, with five or six expanded leaves. At this stage they had a cycle of several adventitious roots which exceeded 10 cm. in length, with a diameter of 1.1–1.8 mm. Uniform sets of plants were selected at 8:00 A.M., the installation and potometric adjustments were completed by 10:00 A.M., and half-hourly readings were made until 3:30 P.M. As in the initial studies (5), it was found desirable to excise all but the test root at the beginning of the experiment, since this helped to eliminate variations due to differences in total absorbing surface. When all roots are left intact, the test root exhibits the same type of gradient as under these conditions but the rate of entry is appreciably slower. At the conclusion of each experimental run the root segments inclosed in the potometric chambers were measured with micrometer calipers and their area determined. Test plants were discarded when there was evidence of injury due to technical difficulties in attaching the potometers.

Three substrates were used: (*a*) the base nutrient at 0.8 atm. osmotic concentration, (*b*) the base nutrient plus added NaCl (48 m.e./l.) to produce 2.8 atm., and (*c*) the base nutrient plus NaCl (96 m.e./l.) to produce 4.8 atm. The actual osmotic concentrations were calculated from freezing-point depression determinations. In order to study the effect of the status of the test root on rate of entry of water, plants were preconditioned in one series of experiments. The concentration of the nutrient solution in which the plants were grown was increased by steps of 0.5 atm. per day to the desired level (2.8 or 4.8 atm.) by adding successive increments of NaCl. The plants were maintained at the final concentration for 5–7 days to permit the roots to attain the required length and become conditioned to the final osmotic concentration of the substrate.

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Investigation

A. Nonconditioned plants

Following the procedure used in the initial experiments (5), roots of nonconditioned plants were tested at the 10-, 6-, and 2-cm. levels in substrates with osmotic concentrations of 0.8, 2.8, and 4.8 atm., respectively. In all substrates the rate of entry was lowest at the 2-cm. level, and in the control (base nutrient) treatment there was a significantly descending gradient of rate of entry from the 10- to the 2-cm. levels in approximately 82% of the plants tested (table 1). Under the 2.8 and 4.8 atm. treatments there was little difference in rate between the 10- and 6-cm. levels, but in both cases it fell sharply at the 2-cm. level. On the basis of the data obtained, it appeared that in practically all cases the zone between the 10- and 6-cm. levels could be regarded as the region of maximum entry of water. For this reason the average of the rates of entry for these two levels under any given treatment was used in some comparisons as being representative of the region of most active absorption (table 2).

When the rates at corresponding root levels under the three treatments are compared, it is evident that increased concentration of salts in the substrate results in a very significant decrease in entry of water (fig. 2). The reduction at

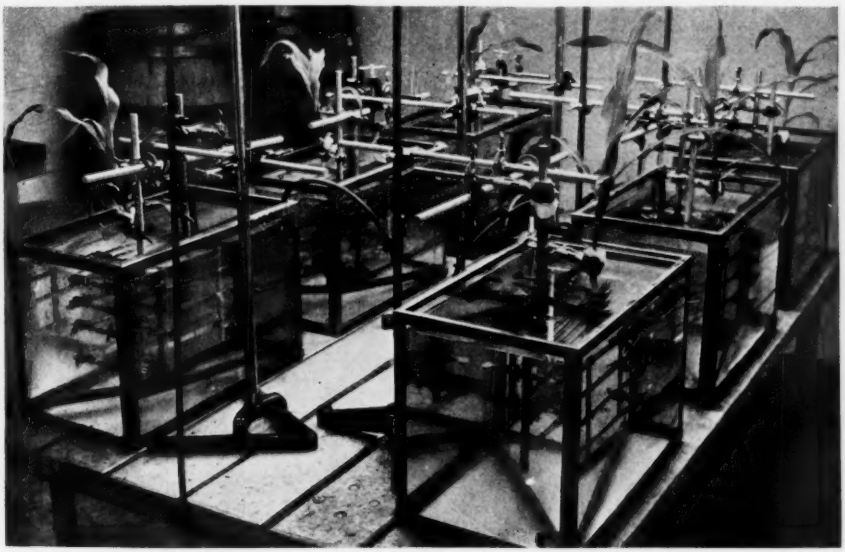


Fig. 1.—Aquaria with test plants in position. Three potometers attached to each test root at 2, 6, and 10 cm. from root tip; fourth potometer is the check.

TABLE 1

Location of maximum rate of entry of water

Treatment	Maximum rate	
	At 10-cm. level (%)	At 6-cm. level (%)
Base nutrient (control)	82	18
2.8 atm. nonconditioned	62	38
2.8 atm. preconditioned	59	41
4.8 atm. nonconditioned	53	47
4.8 atm. preconditioned	33	67

TABLE 2

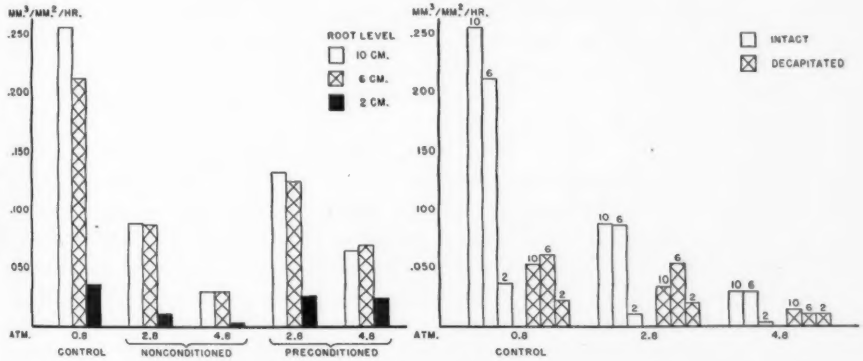
MEAN RATE OF ENTRY OF WATER AND PERCENTAGE DECREASE IN RATE FOR NONCONDITIONED AND PRECONDITIONED ROOTS

Root level	Treatment and osmotic concentration									
	Control (base nutrient) 0.8 atm. os. conc.	Base nutrient + 2 atm. NaCl (total os. conc., 2.8 atm.)				Base nutrient + 4 atm. NaCl (total os. conc., 4.8 atm.)				
	Nonconditioned	Nonconditioned		Preconditioned		Nonconditioned			Preconditioned		
	Entry ($mm.^3/mm.^2/hr.$)	Entry ($mm.^3/mm.^2/hr.$)	Percentage decrease from control	Entry ($mm.^3/mm.^2/hr.$)	Percentage decrease from control	Entry ($mm.^3/mm.^2/hr.$)	Percentage decrease from		Entry ($mm.^3/mm.^2/hr.$)	Percentage decrease from	
							2.8 atm.	Control		2.8 atm.	Control
10 cm.	0.249(19)*	0.075(12)	70	0.102(14)	59	0.030(15)	60	88	0.065(18)	36	74
6 cm.	.211(20)	.086(13)	59	.123(17)	42	.023(13)	73	89	.066(17)	46	69
2 cm.	.038(25)	.010(12)	74	.024(22)	37	.003(13)	70	92	.024(18)	0	37
Ave. 10 cm. + 6 cm.	0.229(39)	0.082(25)	64	0.114(31)	50	0.027(28)	67	88	0.065(35)	43	72

*Figures in parentheses indicate number of roots tested.

each higher level of concentration ranges from 59 to 74%. If the average of the rates at the 6- and 10-cm. levels is used as a basis of comparison, the percentage decrease under the treatments at 2.8 and 4.8 osmotic concentrations is 64% and 67%, respectively. At the highest salt level the rate of entry of water into the root is reduced to 12% of that absorbed under the control treatment (table 2).

ditioned plants, the rate at corresponding root levels was lower in substrates of high osmotic concentration, but the decrease in rate of preconditioned plants was significantly less than that for nonconditioned ones, indicating that the plant has some capacity to adjust itself to high concentrations of salt (10). At 2.8 atm. osmotic concentration the average of the rates for the 6- and 10-cm.



FIGS. 2, 3.—Fig. 2 (left), rate of entry of water into adventitious roots of corn at three levels in substrates of 0.8, 2.8, and 4.8 atm. osmotic concentration. Fig. 3 (right), effect of decapitation on entry of water into corn roots at 10-, 6-, and 2-cm. levels in substrates of 0.8, 2.8, and 4.8 atm. osmotic concentration.

B. PRECONDITIONED PLANTS

Except during the period of germination and seedling development, plants grown in saline soils under irrigation are not ordinarily subjected to such drastic changes in concentration of the substrate as occur when nonconditioned plants are transferred from a nutrient solution of 0.8 atm. osmotic concentration to one at 2.8 or 4.8 atm. For this reason plants were preconditioned as described in the section on Procedure, and the entry of water was determined in the same manner as for nonconditioned plants.

With preconditioned plants there was little difference in rate of entry at the 6- and 10-cm. levels but a sharp drop at the 2-cm. zone (fig. 2). Like the nonconditioned plants, the rate at corresponding root levels was reduced 50% from the control rate, as compared with 64% with nonconditioned plants. At 4.8 atm. concentration the rates for preconditioned and nonconditioned plants were 43 and 67%, respectively, lower than the 2.8 rates; and the rate for preconditioned plants was 72% less than the controls as compared with 88% for the nonconditioned plants (table 2).

With preconditioned plants in the 4.8 atm. substrate, 67% of the test roots had somewhat higher rates of entry at the 6-cm. than at the 10-cm. level (table 1). This shift in the axial gradient of rate of entry appears to be due in part to the relatively advanced maturity at corresponding test levels of the roots precon-

ditioned in substrates of high salt concentration and in part to the concentration of the substrate itself.

It has been pointed out that variations in relative maturity due to differences in rate of growth and in the nutritional status of test plants may materially affect the rate of entry of water and salts (**5, 7, 13**). Histological considerations relative to this situation are discussed in a later section. Although all test plants were grown and preconditioned in the same manner, some differences in their nutritional status resulted from variation in climatic factors, since the studies were carried on during a period of several months.

Because of these and other variables incident to the use of living plants as the test units, the data were subjected to statistical treatment. The homogeneity of the results obtained under each treatment was tested, and a pooled analysis of variance, set up to account for disproportionate frequencies in number of observations, was carried out. The analysis based on the combined average rates of the 6- and 10-cm. levels was computed for the ten possible individual comparisons that can be made between the five treatments. Highly significant differences were found in all but two cases. The difference between the nonconditioned and the preconditioned 2.8 atm. treatments was significant, but there was no significant difference between the nonconditioned 2.8 atm. and the preconditioned 4.8 atm. treatments.

C. Decapitated plants

Investigations of entry of water into roots have led to a distinction between the "active suction" of the root system itself and the "passive suction" transmitted to the roots from the shoot (**9, 10**). Some quantitative studies have been made dealing with the role of the root, per se, in absorption. Rosene (**14**), using intact and excised onion roots, found that "the rates of water influx at contiguous levels of the same root after excision were equal to, greater, or less than before excision." Kramer (**9**) found that "the rate of exudation from detopped root systems was only one to five per cent of the rate of transpiration from similar intact plants." White (**18**), on the other hand, working with excised tomato roots in vitro, demonstrated that root pressure may be an important factor in sap movement, exceeding 6 atmospheres external pressure.

In order to compare the rate of water entry into roots of intact with that of decapitated plants, the tops were excised just above the level at which the adventitious roots emerge. This was done after the potometers were attached and all adjustments had been made. The decapitated plants were then tested in the same substrates as used in the preceding experiments. The quantitative data are presented in table 3.

The results following decapitation indicate a marked reduction in entry rate at all concentrations of the substrate at the 10- and 6-cm. root levels (fig. 3). At both levels there was a greater reduction in the base-nutrient solution than in the substrates of higher osmotic concentration. At all root levels the rate is reduced in both intact and decapitated plants as the concentration of the substrate is increased, but the reduction is much less with the latter. In general, it appears that the lower the rate of entry into the intact plant, the less percentile reduction in rate results from its decapitation. The tendency toward uniformity in rates of entry into intact and decapitated plants at the 2-cm. level suggests that entry at that level is due primarily to the "active"

TABLE 3

EFFECT OF DECAPITATION ON ENTRY OF WATER INTO ROOT

TREATMENT AND OSMOTIC CONCENTRATION OF SUBSTRATE	WATER ENTRY (MM.³/MM.²/HR. AT THREE ROOT LEVELS)						AVERAGE OF 10 AND 6 CM.	
	10 cm.		6 cm.		2 cm.			
	Intact	Decapitated	Intact	Decapitated	Intact	Decapitated	Intact	Decapitated
Control (base nutrient) 0.8 atm.	0.249(19)*	0.052(12)	0.211(20)	0.061(12)	0.038(25)	0.022(12)	0.229(39)	0.057(22)
Base nutrient + NaCl 2.8 atm.	.075(12)	.034 (7)	.086(13)	.053 (7)	.010(12)	.020 (7)	.081(25)	.044(14)
Base nutrient + NaCl 4.8 atm.	0.030(15)	0.014(12)	0.023(13)	0.010(13)	0.003(13)	0.010(13)	0.027(28)	0.012(23)

* Figures in parentheses indicate number of roots tested.

absorption of the root; and that, regardless of the concentration of the substrate, there is a relatively constant and rather low water requirement for the apical portion of the root.

ANATOMY AND HISTOLOGY OF CORN ROOT

Mature adventitious roots of corn consist of a central stele, cortex, and epidermis (1, 4). As seen in transection, the stele has a ring of twenty to forty protoxylem strands alternating with small groups of phloem (fig. 4). There are two or three protoxylem strands to each large metaxylem vessel, and the latter are adjacent to the central pith. The outermost cells of the pith extend between the xylem elements and become thick-walled and lignified at maturity, forming a continuous zone of connective tissue. The outermost layer of the stele is the pericycle, which consists of thin-walled cells.

The cortex is comprised of several layers of parenchymatous cells, the innermost one forming the endodermis. The endodermal cells abut those of the pericycle, and at maturity all (except the passage cells which occur on the same radii as the protoxylem points) develop a U-shaped type of thickening on the radial, end, and inner tangential walls (fig. 5). All the endodermal cells have

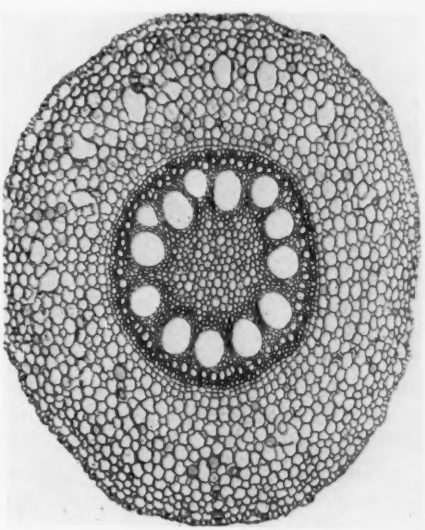


FIG. 4.—Transection of adventitious root of corn at 6 cm. from root tip showing general organization of stele, cortex, and epidermis. Large metaxylem vessels not fully mature but have attained maximum size and are surrounded by lignified connective cells.

Casparian strips, which are more obvious before secondary wall thickening has occurred. The epidermis which forms the

surface layer of the root is ephemeral, disintegrating as the root matures; and the outer layers of the cortex form a lignified and suberized hypodermis.

Microchemical and histological studies of test roots at the 10-, 6-, and 2-cm. levels were made to determine to what extent the observed gradient of entry of water could be correlated with the anatomical status of the root. Freshly cut sections were compared with others that had been fixed, infiltrated with paraffin, and stained with Flemming's triple stain. With the fresh sections Sudan III was used for suberin, phloroglucin-HCl and $KMnO_4$ for lignin, and chlor-zinc-iodide and cuprammonia for cellulose. Resorcin blue gave good differentiation of the Casparian strips.

The progressive development of the tissues of the corn root is illustrated in figures 6–8. The histological changes of most interest in relation to entry of water are those occurring in the vascular, endodermal, and hypodermal tissues. The epidermis does not undergo much change between the 2- and 10-cm. levels, except that there is some elongation of root-hair primordia. The root hairs are shorter and less numerous when the plants are grown in water culture. This should be borne in mind when applying these data to plants growing in soil. Under field conditions, the development of root hairs would be at a peak in the region of differentiation and maturation (6–10-cm. zone), and this would tend to accentuate the gradient of entry of water observed in water-culture studies.

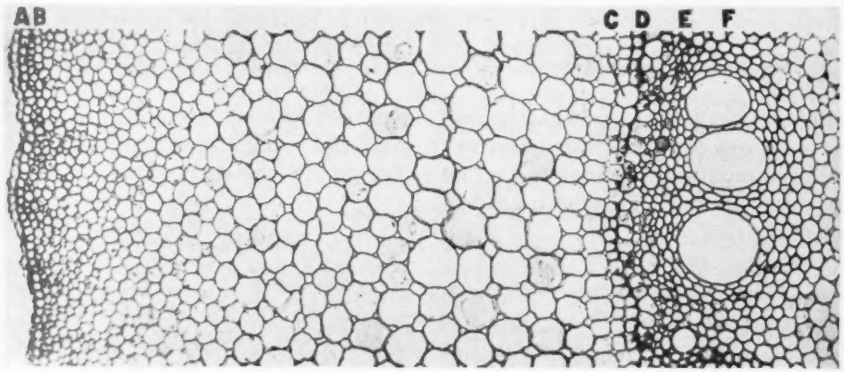
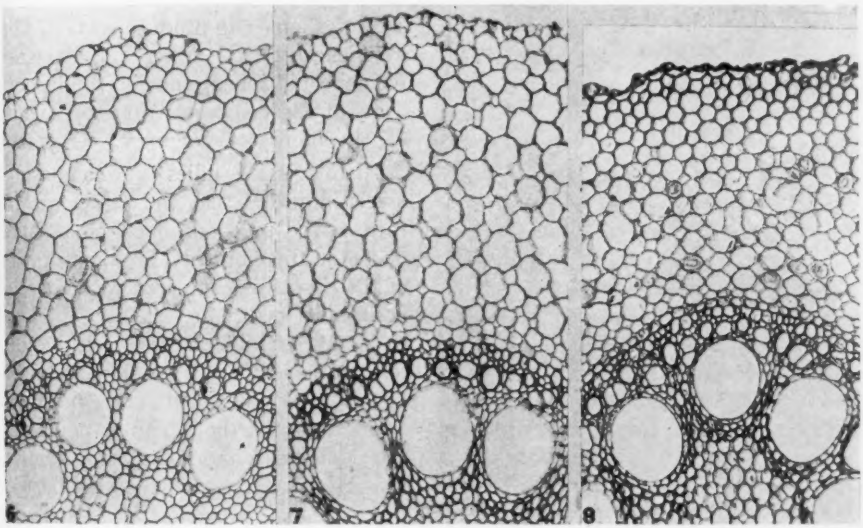


FIG. 5.—Transection of mature corn root showing *A*, disintegrating epidermal layer; *B*, lignified hypodermal cells; *C*, passage cells of endodermis; *D*, endodermal cells with U-shaped thickenings; *E*, mature vascular elements of stele; and *F*, zone of lignified connective tissue.

At the lowest level (2 cm.) at which entry of water was measured, the functional protoxylem elements have thin lignified walls, while the metaxylem vessels are still in process of differentiation, as evidenced by the presence of cytoplasm and nuclei in the vessel segments (fig. 6). The end walls of these segments are still present, so that the large vessels do not function in water transport except as there may be a limited segment-to-segment movement (fig. 9*B*). Under special staining, Casparian strips can be observed on the radial and end walls of the endodermal cells.

At the intermediate level (6 cm.) there is evidence of further lignification of the

protoxylem and advanced maturation of the outermost metaxylem elements. Most of the large innermost metaxylem vessels are probably functional at this level, as the end walls of the vessel segments are partially or wholly resorbed in a majority of cases observed. The radial walls of the hypodermis may show initial suberization, and the Casparian strips of the endodermis are suberized and give radial walls of the endodermis are suberized and partially lignified, but all the endodermal cells are still "passage cells" as far as structure is concerned. Suberization and lignification of the inner tangential walls do not occur until the root is more nearly mature (fig. 5). The walls of some of the hypodermal cells are suberized, and there are indications of the deposition of lignin-like substances.



FIGS. 6–8.—Transections of nonconditioned corn roots showing relative maturation of hypodermal, endodermal, vascular and connective tissues at 2-cm. (fig. 6), 6-cm. (fig. 7), and 10-cm. level (fig. 8). Root photographed at 10-cm. level was slightly smaller in diameter than those shown in figs. 6 and 7. All at same magnification.

some indication of the deposition of a lignin-like substance. A limited number of well-developed root hairs occur (fig. 7).

Vascular differentiation is well advanced at the highest level (10 cm.) of measurement (fig. 8). The protoxylem elements and the metaxylem vessels are completely mature (fig. 9*A*). The maturity of the stele is indicated further by the thick-walled medullary parenchyma, which forms a zone of connective tissue adjacent to the metaxylem vessels. The

The relative maturity of the root at a given level will be modified by the environmental conditions existing during the growing period. When conditions favor rapid growth and elongation, the 10-cm. level will be less mature than when conditions are less favorable and the growth rate is slow. This difference in growth rate creates a variable in test roots, which accounts in part for variations in rate and gradient of water entry under a given set of experimental conditions.

The effect of the concentration of the substrate on rate of maturation of corn roots is evident when they are preconditioned by additions of NaCl to the nutrient solution. If the plants are conditioned in a substrate of 2.8 atm. osmotic concentration, the roots reach the required length more slowly than those grown in nutrient solution at 0.8 atm. concentration, and more pronounced growth inhibition is observed when plants are conditioned to an osmotic concentration of 4.8 atm. In general, it took the plants preconditioned to the 4.8 level 2–4 days longer to produce roots of suitable length for the tests. The diameters of nonconditioned and preconditioned roots at the test levels were not significantly different, averaging 1.33–1.37 mm.

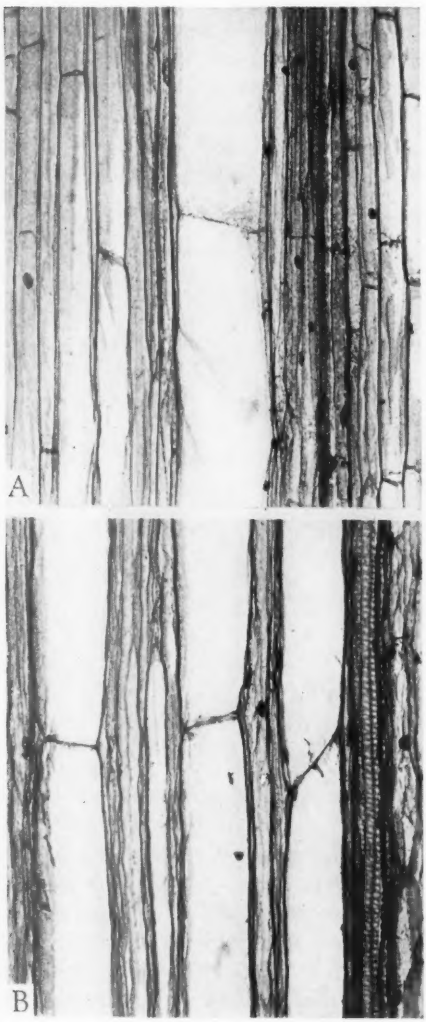


FIG. 9.—Tangential longisections of corn root cut through large metaxylem vessels. *A*, at 10-cm. level; *B*, at 2-cm. level. In *B*, metaxylem vessels are still immature and end walls of vessel segments intact. In *A*, metaxylem vessel has attained full size and end wall of vessel segment almost completely resorbed. Walls of adjacent connective tissue are lignified. Both at same magnification.

Histological studies at comparable levels indicated that the slower growth rate of the preconditioned roots is reflected in the more advanced maturation of the tissues as compared with those of nonconditioned roots. At the 2-cm. level no significant differences in relative differentiation of tissues could be noted between nonconditioned and preconditioned roots; at the 6- and 10-cm. levels the tissues were more mature in the latter. At the 6-cm. level the vascular elements and the connective tissue surrounding them were slightly thicker-walled and more lignified than those of the nonconditioned roots. No outstanding differences in the endodermal and hypodermal tissues were observed.

At the 10-cm. level the preconditioned roots were much more mature than the nonconditioned ones. The secondary walls of the metaxylem elements were thicker, there was more lignification of the endodermis, and suberization of the walls of the hypodermal cells was more advanced. This latter condition may account in part for the shift in the peak of the gradient of water entry to the 6-cm. level which was observed in 67% of the

preconditioned roots at the 4.8 atm. concentration (table 1).

Discussion

The entry of water into roots and its transport to the stele have been ascribed to an osmotic mechanism, to imbibition, to secretory action, or to some combination of such forces. In any event, work must be done and energy supplied to do that work. HENDERSON (6), using corn seedlings in liquid cultures, found a close correlation between the volume of water absorbed and the respiration of the root, indicating an expenditure of energy in the process of absorption. VAN OVERBEEK (17), experimenting with decapitated tomato plants, described an "active" component of root pressure that could be reversibly eliminated by KCN and suggested that it was connected with respiratory processes. GREGORY and WOODFORD (3) found that oxygen intake was greatest in the segment containing the tip of the root of *Vicia faba* and decreased in the next two segments from the tip, but they were not able to demonstrate consistent differences in water uptake in the different segments.

In the present studies with nonconditioned plants, an ascending axial gradient in the rate of entry of water was observed in a great majority of cases when rates at the 2-, 6-, and 10-cm. root levels were compared. Preconditioning, with the consequent retardation in rate of growth, tended to shift the point of most rapid entry toward the apical region of the root, so that frequently the highest rate was recorded at the 6-cm. level. A similar shift occurred owing to high concentration of the substrate in many cases. In all cases the rate was lowest at the 2-cm. level, and the general picture is in agreement with our earlier study (5) and that of ROSENE (13).

The major portion of the water required by a plant enters the root in a zone extending from the level at which differentiation of the xylem elements occurs to the level at which the endodermis becomes entirely suberized (11, 15, 16). But the rate of entry is not equal throughout this zone, and in the adventitious roots of corn the observed rates were well correlated with the relative development of the xylem at the three test levels. At the 2-cm. level, where the lowest rates of entry occurred, only the first protoxylem elements were mature; at the 6-cm. level, where the intermediate metaxylem vessels were also fully differentiated, higher rates of entry occurred. In most cases the largest metaxylem vessels were fully mature at approximately the 10-cm. level, at which point the highest rates were recorded, except in instances where growth in length was retarded by preconditioning in a saline substrate or by unfavorable climatic conditions.

A second limiting histological factor is the status of the cortical and epidermal tissues with respect to suberization and lignification. At the apex of the root the root-cap cells are impermeable to water, but this zone is not more than a few millimeters in extent. PRIESTLEY and TUPPER-CAREY (12) have found that the meristematic tissue at the root apex is relatively impermeable to water under normal conditions. It seems probable that the water requirements of the root apex are less than those of the zone of elongation, which—in addition to requiring hydration for growth—is (together with the zone of maturation above it) the region through which water must be transported to meet the requirements of the shoot.

Above the level of most rapid entry the limiting factor appears to be the suberization of the hypodermis and endodermis. In the corn root there is a progressive suberization of the hypodermal layers. At approximately 4 cm. from the root tip the thin radial and end walls of the hypodermis may be slightly suberized; at the 10-cm. level there is marked suberization of these walls, and in some cases suberization of the inner tangential walls has begun. At higher levels all the walls of the hypodermis—and some of those of the subhypodermal cells—are suberized, and there is some lignification. Paralleling this development, the walls of the endodermal cells become suberized and impregnated with a lignin-like substance. Casparian strips are evident at the 2-cm. level. There are indications of definite suberization and deposition of lignin-like substances on the Casparian strips at the 6-cm. level, which become more pronounced toward the 10-cm. level. Above this point suberization and lignification of the inner tangential walls begin, but the tangential walls of some of the passage cells remain unsuberized, permitting a limited cross-transfer of water.

Thus the histological picture of root ontogeny is correlated with the gradient of water entry, although this gradient may be modified by variations in the rate of growth of the root and the rate of maturation of the vascular, cortical, and epidermal tissues. However, roots of different species exhibit marked differences in their development (4), and it is to be expected that gradients of other types may be found when quantitative measurements of entry are made.

There are few quantitative data that indicate the extent to which the entry of water is restricted when the osmotic concentration of the soil water or nutrient solution is high. EATON (2), using a divided root system technique, found that the entry of water into corn and tomato roots was progressively reduced by adding increments of 10, 20, and 40 m.e./l. Cl. Using nutrient solutions of 0.3 and 1.8 atm. osmotic concentration, he found that the entry of water into corn roots was twice as rapid in the solution of lower osmotic pressure.

Our data based on direct measurement of water entry per unit of surface area per hour indicate a pronounced reduction in rate at all levels when the concentration of the substrate is increased. With nonconditioned plants an increase of 4 atm. in osmotic concentration reduced the rate 88%. When the test plants were gradually preconditioned to the higher osmotic concentrations over a period of several days, the rate of entry was 16% greater than with the nonconditioned plants, indicating some adaptive capacity in this regard.

Salt-tolerance tests at the United States Regional Salinity Laboratory and elsewhere (8) indicate that corn is less tolerant to high salt concentration than many other crop plants. It seems probable that the more salt-tolerant species would show a less pronounced response to high concentration of the substrate in terms of reduced water entry than did corn. It is possible that the relative tolerance of crop plants to saline conditions may depend in large measure upon the rate of water entry when the soil solution has a high osmotic concentration.

The marked reduction in rate of entry which follows decapitation emphasizes the role of the shoot in this process. The loss of water from the shoot by transpiration and its utilization in synthetic processes in the leaves are major factors in determining the rate of entry into the root.

The root of a decapitated plant, through its osmotic mechanism, imbibition, and the expenditure of energy, may obtain water even when the osmotic concentration of the substrate is high, but the rate of entry is low compared with that of an intact plant.

There is much less difference in the rates of entry at the three test levels in decapitated than in intact plants. The difference in rate at the 10-, 6-, and 2-cm. levels with decapitated plants in the base nutrient and the 2.8 atm. solutions is not great and is practically negligible at the 4.8 concentration. The somewhat higher rates at the 10- and 6-cm. levels are probably due to the movement of water to the upper portion of the root and the basal portion of the stem from which the adventitious roots arise. The values obtained at the 2-cm. level indicate that the tips of roots of decapitated and intact plants take in essentially no more water than is utilized locally.

Summary

The entry of water into corn roots was determined quantitatively with a potometric device attached at three root levels: 2, 6, and 10 cm. from the root tip. Substrates of three osmotic concentrations were used—0.8 (control), 2.8, and 4.8 atm., with NaCl as the salt added to the base nutrient solution. Tests were run with nonconditioned, preconditioned, and decapitated plants.

1. In general the most rapid entry occurred in the zone between the 6- and 10-cm. levels above the root tip. In all tests the rate of entry was lowest at the 2-cm. level, and 82% of the control plants exhibited the highest rate at the 10-cm. level.

2. The state of maturity of the vascular tissue affects the rate of entry. The differentiation of the water-conducting elements is progressive, and the full complement of vascular tissue is not functional at points below the level of most rapid entry.

3. The rate of entry increases as the epidermal cells mature. It decreases as they disintegrate and the hypodermal and endodermal cells become suberized and lignified.

4. Substrates of high osmotic concentration tend to inhibit meristematic activity and elongation of the root. Under these conditions the zone of most rapid entry is nearer the root tip.

5. With both nonconditioned and preconditioned plants, high osmotic concentration of the substrate resulted in a significant reduction in the rate of entry.

6. At corresponding root levels, and in substrates of equal osmotic concentration, preconditioned plants had higher rates than nonconditioned ones.

7. When compared with intact plants, decapitated ones exhibited a marked reduction in entry rate in all substrates and at all root levels except the 2-cm. level in the high salt solutions.

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GROWTH AND DEVELOPMENT IN RANGE GRASSES. III. PHOTOPERIODIC RESPONSES IN THE GENUS BOUTELOUA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 556

CHARLES E. OLMSTED

Introduction

Since only limited studies (**4, 16**) on photoperiodic responses of native range grasses have been made, in contrast with more extensive work on cultivated forage grasses and cereals (**2, 3, 6, 7, 8, 17, 23**), and because there were indications (**4, 16, 18, 21, 25**) that some species in the genus *Bouteloua* might be photoperiodically sensitive, it was felt that such an investigation of this genus might be timely.

The genus is of great economic importance in the western half of the United States, where several of its species contribute largely to the forage supply on many native ranges, and where some of them show great promise in artificial reseeding of denuded range lands (**11, 13, 24, 27**). It is a rather sharply defined—although diverse—taxonomic unit, the species falling into two subgenera (**11, 12**). The various species show considerable differences in distribution and in adaptation to environment. Recent investigations (**10, 14, 15, 19, 21, 22, 24**) have emphasized a morphological, physiological, and cytological diversity within certain individual species. Some of these facts suggested that a knowledge of photoperiodic responses in the genus might contribute to an understanding (*a*) of the factors limiting the geographical distribution of the different species, and (*b*) of those affecting vegetative and reproductive habits during the growing season, both for the species under investigation and for the grass life-form in general. Such knowledge (*c*) should be of value in showing whether photoperiodic requirements need be considered in breeding programs aimed to produce desirable strains for reseeding and (*d*) might contribute to further understanding of the evolution of photoperiodic responses within taxonomic units.

Experiments were initiated in the spring of 1941 at the University of Chicago and have been continued since. These experiments were the outgrowth of earlier ones conducted at the University of Arizona on drought responses of *B. curtipendula*, in which certain unexpected results were obtained in plants grown with supplemental illumination during the winter months (**18**). The present paper is concerned with the photoperiodic responses of one strain each of six species of the genus, as shown by survey experiments conducted in 1941 and 1942. On the basis of these results, other experiments, now in progress (**15, 19**), were initiated along more specific lines. They will be reported on at a later date.

Material, methods, and environmental conditions

The six species investigated in 1941 were side-oats grama (*Bouteloua curtipendula*), slender grama (*B. filiformis*), hairy grama (*B. hirsuta*), Rothrock's grama (*B. rothrockii*), black grama (*B. eriopoda*), and blue grama (*B. gracilis*). The first two species are in the subgenus Atheropogon, and the others in the subgenus Chondrosium. Spikelets of the first three species, collected on the Santa Rita Experimental Range in southern Arizona, were obtained through the courtesy of the Southwestern Forest and Range Experiment Station and Dr.

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

ROBERT DARROW of the University of Arizona. Caryopses of Rothrock's grama from Sells, Arizona, and of black grama from Pima, Arizona, were obtained through the kindness of Mr. L. P. HAMILTON of the Soil Conservation Service, Tucson; while Mr. LEON HURTT of the Northern Rocky Mountain Forest and Range Experiment Station, Missoula, Montana, furnished caryopses of blue grama from Silver Star, Montana. Thus material of five of the six species originated within a fairly small region in southern Arizona under similar natural photoperiods, while that of blue grama came from a source about 13° in latitude north of the others and thus from an area with considerably different natural photoperiodic conditions.

Side-oats, hairy, and blue grama are species of wide distribution in western United States, extending south into Mexico or even farther. Blue and side-oats grama range into Canada, and hairy grama occurs in South Dakota. Over their entire ranges these species are subjected to widely differing conditions of maximum day length in the growing season, especially when contrasted with the other three species (slender, black, and Rothrock's grama), which occur in the United States only in southern New Mexico, Arizona, southwestern Texas, or southern California, extending south into Mexico or South America (**11, 12**).

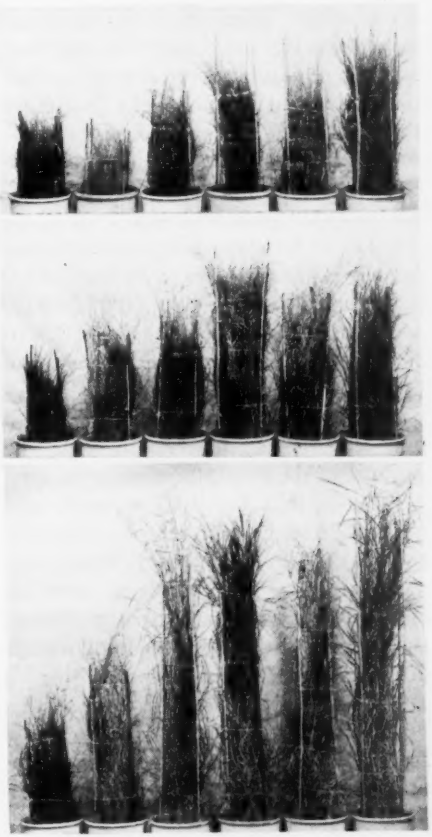
One hundred eighty 2-gallon glazed crocks, 8 inches in diameter and 10½ inches deep (inside measurements), with rounded bottoms provided with a center drain, were uniformly spaced on six trucks (30 per truck) which could be rolled into ventilated lightproof photoperiodic sheds. The crocks were evenly filled and packed with a sandy loam soil of prairie origin (near Chicago) and of medium fertility. It proved to be favorable for all six species. On April 6, 1941, uniform caryopses were planted 1 cm. deep at evenly spaced intervals at the rate of 25–35 per crock (150–200 for *B. rothrockii*). Thirty pots were planted to each species, five on each of the six trucks. To compensate for possible positional effects, the crocks were at first systematically distributed on the trucks, but it later proved necessary to arrange them so that the taller species did not shade the shorter ones. Positional effects were found not especially important.

Immediately after prompt and uniform (except in *B. eriopoda*) germination, the plants on the three trucks in each of the two adjoining rooms were subjected to photoperiods of 8, 12, and 16 hours. Trucks on 8- and 12-hour photoperiods received natural daylight between 8:00 A.M. and 4:00 P.M. (CDT) each day in the greenhouse but were rolled into the lightproof sheds for the remainder of the 24 hours. Trucks on 12-hour photoperiod also received 4 hours of artificial light from 4:00 to 8:00 P.M. Trucks on 16-hour photoperiod remained in the greenhouses continuously and received supplementary illumination after sunset throughout the season. Supplementary illumination over each truck was provided by three 200-watt Mazda lamps mounted in individual reflectors. Light intensity from this source at pot-level varied from 100 to 180 foot candles (Weston Sunlight meter), depending on position and plant height. The lights were raised as the plants grew to maintain this intensity at approximately average foliage height.

Natural daylight intensity on bright clear summer days in the greenhouses at Chicago commonly ranges up to 5000–6000 foot candles—occasionally even more. While these maximum values are much lower and are attained much less frequently than are the values of 10,000

foot candles or more in the natural distributional areas of the species under consideration (5), the relatively vigorous growth of all species (figs. 1–3) suggested that the lower light intensity at Chicago was not an important determining factor in the responses observed during the spring, summer, and early autumn months. It is probable that it is more favorable for growth in height than are the higher intensities (cf. BENEDICT, 5). In late autumn and winter at Chicago, however, with much lower intensities of natural light, most species became practically dormant, at least so far as growth and flowering were concerned. Some recent experiments with artificial supplemental light indicate that the low natural light intensity is the chief factor involved in this dormancy or limited growth in warm greenhouses in the winter months. For this reason, experiments on photoperiodic responses in these grasses have been conducted chiefly in the late spring and summer months.

Heating and ventilation of the greenhouses was so regulated as to minimize temperature differences among the various treatments and to provide favorable conditions for growth. The daily record from maximum-and-minimum thermometers placed on each truck and the growth observed indicate that the minor temperature differences were of little significance in affecting the photoperiodic responses. While the temperature values at Chicago were undoubtedly somewhat different from those in the native habitats, the photoperiodic responses are sufficiently fixed as not to be affected appreciably within the range of temperatures favorable for growth likely to be encountered by the species during their active periods, especially for the five strains from Arizona. Subsequent experiments have indicated that winter chilling is unnecessary to bring about reproductive activity in these strains. The results may probably be interpreted rather generally, therefore, although it is realized that photoperiodic responses are conditioned by numerous environmental factors (4, 6, 17, 20, 23), each of which might have different values in natural environments from those encountered in these experiments.



FIGS. 1–3.—Ten plants per pot 130 days after germination of (left to right) *Bouteloua gracilis*, *B. eriopoda*, *B. hirsuta*, *B. filiformis*, *B. rothrociki*, and *B. curtipendula:* fig. 1 (top), grown on 8-hour photoperiod; fig. 2 (middle), 12-hour photoperiod; fig. 3 (bottom), 16-hour photoperiod.

Favorable soil-moisture conditions were maintained at all times and nutrient levels in the soil were apparently adequate. Seedlings were thinned to ten per pot after the first month of growth, and while those remaining were selected somewhat for uniformity in size and spacing, the 100 plants of each species thus subjected to each light treatment through the summer probably represented a reasonably adequate and accurate sample of a population obtainable from the particular seed sources. In a few cases a slightly lower number than ten per pot was available because of insect injury in the early period of growth, and—in the case of *B. eriopoda*—because of uneven germination and growth in the first month (April), apparently due in part to a somewhat higher temperature requirement than in the other species. Because of the general similarity in competition within the various pots, the range in photoperiodic response among the 100 individuals (or less) of one species under similar light treatment must be ascribed more to genetic variation within the population than to lack of uniformity in the environment.

It became necessary, because of the luxuriant growth of the taller species, especially on 16-hour photoperiod, to provide supports of bamboo and twine for the tops. This inadequacy of the culms could not be ascribed to etiolation in response to low light intensity at Chicago, since they could easily have supported the foliage ordinarily produced by the various species, but rather it seemed to be due to the continuation of vegetative growth beyond the limit reached under natural environment.

Aside from general notes throughout the season, extensive data were collected, especially on flowering responses, during June when the plants were 2–2½ months old. Late in August and early in September, when the plants were 4½–5 months old, three pots (five for *B. eriopoda*) of each species from each truck were harvested by clipping the tops approximately 1 inch above the soil surface. Thus tops of approximately 60 plants (100 for *B. eriopoda*) of each species under each treatment were available for study. The roots and crowns were washed out of three of the harvested pots (four for *B. eriopoda*) of each species for each treatment at this time and saved for examination and dry-weight determination. The other three pots (six for *B. eriopoda*) of each species for each treatment were returned to the trucks and reciprocally transferred, so that for the ensuing period three pots of clipped plants of each species (six of *B. eriopoda*) were placed under each of the photoperiods, two of the three (four of the six of *B. eriopoda*) having previously grown under the other photoperiods. This transfer was made to see whether any "carry-over" effect of previous treatment remained in the crowns and roots of the clipped plants. Simultaneously, similar reciprocal transfers were made among the four unharvested pots of each species on similar treatment (except none for *B. eriopoda*), two pots of each species and treatment being left on the same treatment, while one was transferred to each of the other two conditions. In addition to notes during the ensuing growth period, data were obtained on these plants when the tops of those previously unclipped were harvested in early December, those of the remaining pots in middle February, 1942.

Most of the pots were then discarded, except those of *B. filiformis* and *B. curtipendula*, which were retained for experimentation the next summer (1942), but were placed on natural photoperiod in the ensuing interval until July 22,

1942. These latter plants resumed relatively vigorous growth in March, after being clipped in February, and many inflorescences were produced under natural photoperiod in late April and early May. For the remaining 2 months of long natural photoperiods until July 22, only vegetative tillers were produced. On that date all plants were clipped to within 1 inch of the ground, and a variable number of pots of each of the two species was placed under photoperiods varying by ½-hour intervals from 13 to 15½ hours, in an effort to determine more precisely the critical light period for these species in their second year of growth. For the six treatments, 9 hours of natural daylight were provided (8:00 A.M.–5:00 P.M.), supplemented by artificial illumination after 5:00 P.M. to provide total photoperiods of 13, 13½, 14, 14½, 15, and 15½ hours. Supplemental illumination by Mazda lamps was given under frames covered with lightproof black cloth, at intensities of 50–100 foot candles, depending on plant height. Flowering responses were observed for the next 2 months. On September 21, the plants of *B. filiformis* were again clipped and data taken on the tops. The same was done with *B. curtipendula* on October 16. These pots were then returned to natural photoperiod.

Results

According to the recent definition of ALLARD and GARNER (3), and from the data in table 1 and figures 4 and 5, the five species with strains from Arizona, especially side-oats, slender, hairy, and Rothrock's grama, should probably be considered short-day plants, while blue grama from Montana falls—although not so precisely—into either long-day or indeterminate categories. The former failed to flower, or were delayed in flowering, on 16-hour photoperiod, in contrast with responses on 8- and 12-hour photoperiods; while the latter, although flowering, first on the shorter light periods, showed more rapid acceleration of flowering and eventually flowered more profusely on the longest photoperiod. In general, the five species represented by southern strains exhibited more rapid and vigorous flowering responses on 12-hour than on 8-hour photoperiod, suggesting better adaptation to the photoperiod nearest that under which they naturally flower in late July and August in Arizona, although not falling clearly into the intermediate group defined by ALLARD (1).

TIME REQUIRED FOR FIRST FLOWERING

The beginning and progress of flowering was measured by the emergence of recognizable inflorescences from the surrounding leaf sheaths. While this is a less accurate measure than are dissection methods (6, 9), which enable one to recognize spikelet initiation in the apical meristem, the time and materials available did not permit their use. Moreover, observations with these methods have indicated that development of inflorescences is very rapid in these forms and that initiation may occur after marked internodal elongation of the culm, in contrast with certain other grasses (9).

FLOWERING IN YOUNG PLANTS.—Table 1 shows the number of days after germination on which the first inflorescence appeared in each species on each treatment. The data here and elsewhere suggest a somewhat fundamentally different behavior of the two species, side-oats and slender, belonging to the subgenus Atheropogon, from that of the others. The lag in onset of even limited flowering on 16-hour photoperiod was greatest in them. All species flowered first on 12-hour photoperiod, or simultaneously on 8- and

12-hour light periods in this first season of growth from seed. All species flowered in less than 2 months after germination under some one of the treatments (Rothrock's grama in 30 days), in contrast with development in native environments, where this phase is often not reached until the second year or later.

CLIPPED RECIPROCAL TRANSFERS.—Comparable results were obtained after clipping and reciprocal transfers among treatments, in the autumn of 1941. Sideoats and slender grama then failed to flower on 16-hour photoperiod, irrespective of previous photoperiodic treatment, while they first showed inflorescences on the 27th and 18th day, respectively, after clipping when on 12-hour photoperiod, and on the 33rd and 18th day after clipping when on 8-hour photoperiod. There was a slight but not marked lag in the onset of flowering in those plants which, on 16-hour photoperiod prior to clipping, were then transferred to the shorter photoperiods. Any carry-over effect of previous treatment was very limited, however, and was not sufficient after clipping to result in flowering of plants which were transferred from the shorter to the longest photoperiod. Slender grama is obviously capable of very rapid response to the photoperiodic stimulus, a period of 18 days after clipping being sufficient for renewed growth with emergence of inflorescences. In the other three species with strains of southern origin, some flowering occurred on all treatments after clipping and transfer. All pots of hairy and Rothrock's grama flowered during the first month after clipping when on 8- or 12-hour photoperiods, but required 2–3 months on 16-hour photoperiod. Black grama did not grow well after clipping, many plants dying and others which had bloomed before clipping fail-

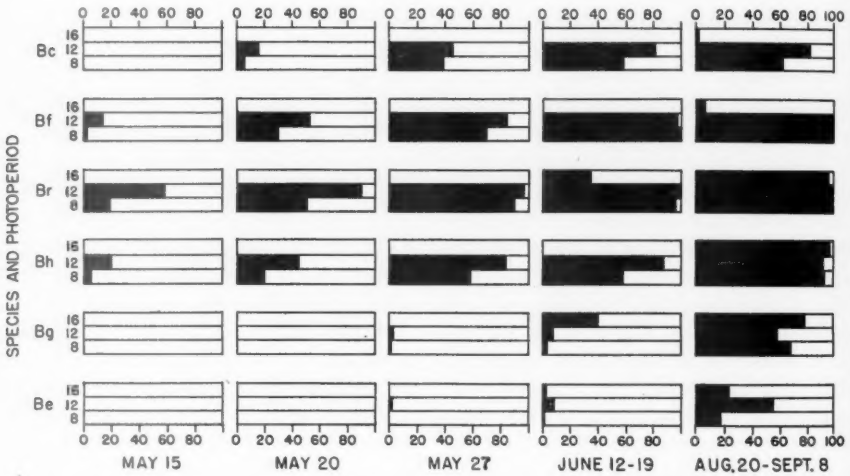


FIG. 4.—Percentages (shaded) of plants in flower (inflorescence emergent above surrounding leaf sheath) of six species of *Bouteloua*, subjected to photoperiods of 16, 12, and 8 hours per day after germination on April 6. Data for May based on 100 or more plants per species on each treatment; for June and August on 60 plants (100 for *B. eriopoda*) per species on each treatment. *Bc, B. curtipendula; Bf, B. filiformis; Br, B. rothrockii; Bh, B. hirsuta; Bg, B. gracilis; Be, B. eriopoda.*

ing to flower. Flowering did occur in the first 2 months on 8- or 12-hour photoperiods, but only in the third month on 16-hour photoperiod. Blue grama from Montana flowered only on 16-hour photoperiod after clipping, possibly confirming its long-day status, but also suggesting that too low light intensity, shown by BENEDICT (5) to be very unfavorable to this species in its flowering behavior, may also have been partly responsible. BENEDICT (4) had earlier classed blue grama as an indeterminate species and indicated that it also required high temperatures to flower, although he did not state the geographic source of his plants.

CRITICAL PHOTOPERIOD.—In the critical-photoperiod experiment on side-oats and slender grama in the summer of 1942, in which 2nd-year plants were distributed among six photoperiods after clipping to the ground, slender grama flowered in 24 days on 13-hour photoperiod, in 28 days on $13\frac{1}{2}$ hours, and between the 28th and 38th days on 14 hours. It failed to flower in 61 days on $14\frac{1}{2}$-, 15-, and $15\frac{1}{2}$-hour photoperiods. Under similar treatment, side-oats grama flowered between the 41st and 61st days on $13\frac{1}{2}$- and $14\frac{1}{2}$-hour photoperiods but failed to flower on the others in 86 days. Under the conditions of this experiment, in which all plants received 9 hours of natural daylight daily, the upper critical photoperiod for these two species lies between 14 and $14\frac{1}{2}$ hours. This is approximately the maximum photoperiod to which they are subjected during active growth in southern Arizona. Failure of side-oats grama to flower on 13-hour photoperiod suggests that this strain, under certain experimental conditions, would fall into ALLARD'S (1) intermediate class, although the other experiments do not bear this out, since the same plants flowered on 8-hour photoperiod in their first year of growth. This indicates the need for caution in interpreting the results of single experiments on limited material and suggests that the range of photoperiods optimum or even favorable for flowering in this species may be much narrower under somewhat adverse conditions, such as the limited duration of high light intensity (9 hours for all treatments) in this experiment, than under more favorable conditions. This is borne out by its failure to flower again on natural photoperiod, although resuming vegetative growth after clipping in October, 1942, until both photoperiod and light intensity apparently became favorable in April, 1943. Slender grama plants, grown under 13, $13\frac{1}{2}$, and 14 hours in the critical-photoperiod experiment, flowered again in 25, 22, and 22 days, respectively, after clipping in late September when placed on natural photoperiod. Plants previously on longer photoperiods ($14\frac{1}{2}$, 15, $15\frac{1}{2}$ hours) did not flower for over 40 days after clipping, suggesting a more pronounced carry-over effect of previous treatment than in the clipping experiments in 1941. All slender grama plants

TABLE 1

NUMBER OF DAYS UNTIL FIRST FLOWERING,* AFTER GERMINATION ON APRIL 6, 1941, OF SIX SPECIES OF BOUTELOUA ON PHOTOPERIODS OF 8, 12, AND 16 HOURS PER DAY

SPECIES	PHOTOPERIOD		
	8 hours	12 hours	16 hours
B. curtipendula	44	42	114
B. filiformis	39	39	106
B. rothrockii	30	30	57
B. hirsuta	39	36	80
B. gracilis	51	51	63
B. eriopoda	>51<74	51	>51<74

* Measured by emergence of inflorescence from surrounding leaf sheath.

continued to flower on natural photoperiod through autumn, winter, and spring months, until the longer photoperiods of late spring became unfavorable, after which they grew vegetatively. Side-oats definitely requires higher light intensities for both satisfactory growth and flowering than does slender grama.

Rate of flowering

Experiment, Summer 1941.—The previous discussion is based primarily on the length of time required for the first plant to flower in each species and treatment. Results statistically more significant are based on the sequence of flowering of all plants in each population (fig. 4). Data are presented of the percentage of plants which were flowering or had flowered (at least one inflorescence emerging or emergent per plant) on five different dates, ending with the harvest in late August and September. They confirm the previous conclusions, that these strains of all species (except blue grama) should probably be regarded as short-day plants, either because a very low percentage flowered on 16-hour photoperiod or because plants which ultimately flowered were delayed on 16-hour photoperiod as compared with the other treatments. Side-oats, slender, Rothrock's, and hairy grama attained nearly their final percentages of plants flowering on the short photoperiods before any flowering occurred on the 16-hour photoperiod, even though in the latter two species practically all plants flowered, while in the first two species flowering was limited to very few plants on 16-hour treatment. Black grama did not follow this general pattern, the plants on 12-hour photoperiod continuously showing the highest percentage in flower, so that at harvest more than twice as many of them had flowered as in the other treatments which showed similar and low values. In blue grama, which flowered first on 12- and 8-hour photoperiods, the percentage of plants in flower on 16-hour photoperiod rapidly increased (data for May 27 and June 12–19), and ultimately this latter treatment had the highest value, although the final data suggest an indeterminate effect. All the data also indicate considerable variation in all species on each treatment as to time of first initiation of inflorescences in individual plants. This was more marked on the shorter than on the 16-hour photoperiod. In fact, at harvest all types of morphological variation were less marked in the latter treatment, especially in side-oats, slender, Rothrock's, and hairy grama. The longer period of strictly vegetative growth for the plants on 16-hour photoperiod permitted more uniform growth, contrasting with the greater difference in time of floral initiation by the various individuals on the shorter photoperiods, which tended to accentuate vegetative differences within each species.

Clipped reciprocal transfers.—Rate of flowering in the clipped reciprocal transfer experiment followed the same general tendencies shown in figure 4, except that side-oats and slender grama failed to flower on 16-hour photoperiod, while blue grama did not flower on 8- and 12-hour photoperiods. Percentages of plants which ultimately flowered in other species and treatments were mostly lower than those shown in figure 4, the percentages for Rothrock's and hairy grama on 16-hour photoperiod proportionately much more so than on 8- and 12-hour treatments. Delay in initiation on the 16-hour photoperiod caused the flowering period to coincide with the season of unfavorable light intensity and limited growth in general, which finally termi-

nated the flowering responses of all species except slender grama.

UNCLIPPED RECIPROCAL TRANSFERS.—Most obvious responses with these transfers were obtained with side-oats and slender grama previously grown on 16-hour photoperiods and transferred to the shorter light periods. In slender grama, all plants—few of which had flowered earlier—initiated inflorescences within 3 weeks after the transfer, either at the tips of elongated vegetative culms and branches from the upper nodes of the latter, or from tillers which were unelongated previous to transfer. In side-oats grama similar results were obtained, although the time required was longer (4–6 weeks) and the percentages of plants originally nonflowering which ultimately flowered were smaller. In this species the inflorescences more generally appeared on new basal tillers. After the reciprocal unclipped transfer of these two species from shorter to long photoperiod, the appearance of new inflorescences ceased in about 2 weeks. In slender grama, vegetative branches from old flowering culms then developed from buds on their upper nodes, these branches overtopping the tips of the old inflorescences. Similar vegetative branches developed in side-oats grama, although the elongation of basal, previously unelongated tillers was more common. The latter occurred in slender grama also. In the similar transfers of Rothrock's and hairy grama from short to long photoperiod there was a delay in inflorescence initiation on subsequently formed tillers, while in the reciprocal transfer this initiation was accelerated. The results on blue grama cannot be summarized, and there were no unclipped transfers of black grama. Unclipped plants of all species left on previous treatment when others were transferred showed a continuation of the pattern already indicated in figure 4 for their earlier growing period. All continued to flower or failed to flower, as at the time of harvest of the other pots, and served to verify the interpretations of the effect of transfers already given.

GROWTH IN HEIGHT

As is generally true in short-day plants, maximum heights were positively correlated with length of photoperiods to which the plants were subjected. Maximum height of a clipped plant was measured from the cut stem ends to the tip of the longest outstretched leaf or to the tip of the inflorescence, whichever gave the greater value. Although the values given are therefore slightly higher than the average standing heights of the plants, they are in proportionate agreement (figs. 1–3, 5). The most striking features of this response were the much greater heights attained by all species on 16-hour photoperiod, whether flowering or nonflowering, than they reach under natural conditions in the field. In figure 5, mean values are given for all plants (solid bars) and for those plants which flowered (solid bars plus hatching) in each species on each treatment. Similar proportionate values were measured earlier in the growing season, differences being observable in the amount of elongation of the first and second foliage leaves in the first week of growth. Length and width of leaf blades and sheaths, length of internodes, and diameter of stems and roots were much greater in the plants on 16-hour photoperiod than in the others. While the dry-weight yield (fig. 6) was therefore much greater in the plants on long photoperiod in four of the species, the forage would have been much less palatable. No effort was made to correlate these responses

with internal anatomical conditions, as STUCKEY (26) has recently done with orchard grass.

Unclipped plants of slender and side-oats grama, left on 16-hour photoperiod after most plants were clipped in August, continued to grow vegetatively and to increase in length, reaching average heights of 130 and 176 cm. by mid-December, when they were harvested. Untallest on 16-hour photoperiod at the time of harvest in February, 1942. Heights were much shorter than in the August harvest, however, especially in black grama, probably because of the less favorable light intensity. There was little obvious effect of previous photoperiodic treatment on the height attained in the various pots of each species on each photoperiod after clipping and

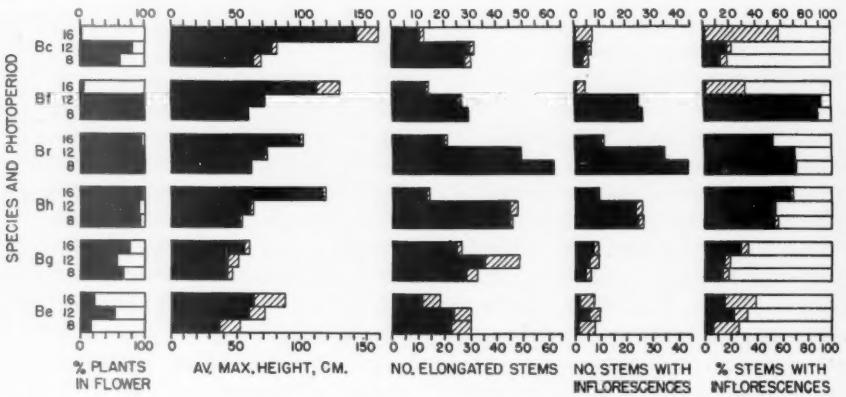


FIG. 5.—Growth and flowering of six species of *Bouteloua*, subjected to photoperiods of 16, 12, and 8 hours per day after germination, and harvested when 4½–5 months old. Solid black bars are averages of 60 plants (100 for *B. eriopoda*) for each species on each treatment; solid black bars plus hatching are averages of all plants which flowered on each treatment: *Bc, B. curtipendula; Bf, B. filiformis; Br, B. rothrockii; Bh, B. hirsuta; Bg, B. gracilis; Be, B. eriopoda.*

clipped plants, previously on 16-hour photoperiod, grew only slightly in length after transfer to shorter light periods, the increase being mainly due to elongation of inflorescences which initiated. Simultaneous transfers of unclipped slender and side-oats grama from 8- and 12-hour to 16-hour treatments resulted in average height gains of 20–40 cm. over their values in August, in contrast with little increase in plants allowed to remain on short photoperiods.

Height growth in the clipped reciprocal transfers confirmed the results of the earlier experiment, all species being transfer, except in slender and side-oats grama. Plants of these species remaining on 16-hour treatment were taller than those transferred to it from shorter photoperiods.

TILLER DEVELOPMENT AND CULM ELONGATION

Internodal elongation of primary axis and individual tillers apparently began sooner in all species on the shorter photoperiods than on the 16-hour light period. Their growth was also terminated much sooner, either as a consequence of obvious development of inflorescences, or without

it, than on the long photoperiod. Although internodal elongation is sometimes taken as the first easily observable evidence of reproductive activity in grasses, it need not be accompanied or soon followed by initiation of spikelet primordia. Recent observations on side-oats grama have shown that vegetative development of such axes may continue from the apical meristem for more than elongation. The August data are averaged on a plant basis for all plants, and for flowering plants only (fig. 5). Fewer tillers with elongated internodes were produced by the plants on 16-hour photoperiod in all species than on the other treatments. The differences in such numbers between the 8- and 12-hour treatments are not statistically significant, except in Rothrock's and blue grama, the

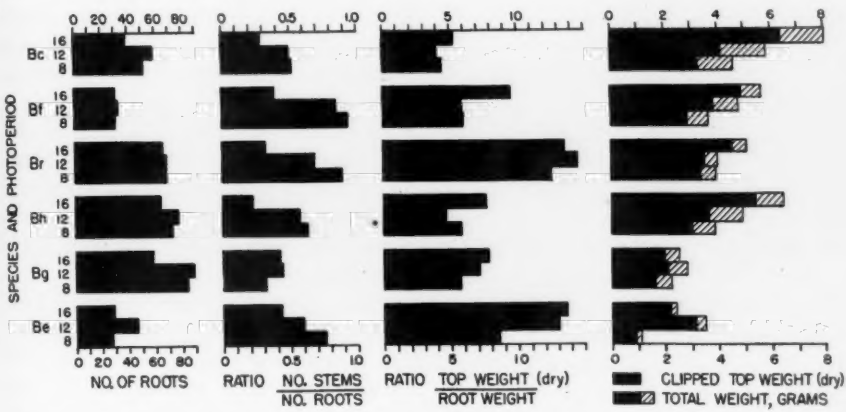


FIG. 6.—Growth of six species of *Bouteloua*, subjected to photoperiods of 16, 12, and 8 hours per day after germination and harvested when 4½–5 months old. Averages of 30 plants per species on each treatment. *Bc, Bouteloua curtipendula; Bf, B. filiformis; Br, B. rothrockii; Bh, B. hirsuta; Bg, B. gracilis; Be, B. eriopoda.*

a year without any suggestion of development of inflorescences, these axes reaching lengths of more than 2 meters.

In the 1941 experiments no data were obtained on the total numbers of tillers formed in the various species and treatments, although they were much the lowest in plants on 16-hour photoperiod. Tillers with one or more elongated internodes were counted on all plants harvested in August, however, and also on previously unclipped slender and side-oats grama harvested in December. These were counted by stripping away the surrounding leaf sheaths to verify the former showing the greatest number on 8-hour photoperiod, the latter on 12. The cessation of height growth of individual axes on the shorter photoperiods resulted in continued production of basal tillers, while the apical meristems remained active and dominant for longer periods on 16-hour photoperiod, so that the rate of basal tiller initiation and elongation was much slower.

New tillers developed throughout the season on all species and treatments. For example, the number of elongated culms doubled in slender and side-oats grama from August to December, 1941, on all

treatments in those plants which were neither clipped nor transferred to a different photoperiod. Tiller counts on clipped plants in February were of little value, since differential death of plants or parts of their crowns after the August clipping confuses the results, but the same tendencies are suggested.

Development of inflorescences

The numbers of stems per plant arising from the crown and on which one or more inflorescences had been developed by August and September are shown in figure 5, on both a total-plant and flowering-plant basis, together with the total percentage of elongated stems which had formed inflorescences. Both sets of data indicate a much more profuse flowering tendency of slender grama on 8- and 12-hour photoperiods, and of Rothrock's and hairy grama on all photoperiods, at the time of the August harvest than in the other three species, when measured by the number of stems involved. Similar results were obtained in the other experiments. The data are not absolute values for the total numbers of inflorescences produced. Individual tillers in the cases of side-oats, Rothrock's, black, and especially slender grama, branched to give rise to more than one inflorescence (up to fourteen on a single tiller in slender grama). This condition was not found in hairy or blue grama. In general, the total number of stems bearing inflorescences was largest in all species on one of the shorter photoperiods. Blue grama is an exception, in that the value for plants on 16 hours of light per day approximately equaled that for those on 12 hours. When based on all plants, the percentages of stems flowering per plant were greater on the short photoperiods for side-oats, slender, Rothrock's, and black grama, whereas they were higher on 16-hour photoperiod for hairy and blue grama. When based on flowering plants only, the flowering response seems most vigorous by this measurement on the 16-hour photoperiod for all species except slender and Rothrock's grama. However, the numbers of side-oats and slender grama plants which flowered on 16-hour photoperiod, and those of black grama which flowered on 8- and 16-hour photoperiods, were so few that probably little significance should be attached to the results. They suggest that when an individual plant is able to flower on all photoperiods, the 16-hour light period may be more favorable for profuseness of flowering than the others.

A similar conclusion is made with more validity on comparing the length of inflorescence, the number of spikes per inflorescence, the length of spike and number of spikelets per spike, and the percentage of sterile florets and spikelets, among all the plants which flowered on all treatments. In all species, observations on these criteria, especially in June and August, indicated a somewhat greater favorability of the 16-hour photoperiod for vigorous flowering. This conclusion need not negate the general short-day status of all species except blue grama. It suggests, however, that conditions of the experiment were not very favorable for continued growth of inflorescences which were more rapidly initiated on the shorter photoperiods. The limitation of 8 hours of natural daylight on both 8- and 12-hour photoperiods, in contrast with the full natural photoperiod available to plants on 16 hours of light, may account in large measure for this response, as well as for less vigorous vegetative growth. In nature the five species with strains from Arizona mature their inflorescences on a decreasing photoperiod of high light in-

tensity in July and August, although it is still in excess of 8 or 12 hours when they are mature.

The contrast in vigor of spikes between the 8- and 16-hour treatments was most marked in hairy grama. On 16-hour photoperiod, five spikes per inflorescence were sometimes produced, ranging in length up to 2 inches and bearing spikelets almost to the tip of the rachis, so that the sterile rachis tip often taken as diagnostic of this species (12) was practically obscured. In contrast, on 8-hour photoperiod, inflorescences occasionally consisted of only one poorly developed spike having few spikelets and a rachis extending $\frac{1}{2}$ inch or more beyond them. In extreme cases no spikelets were developed, a spike consisting of a sterile rachis $\frac{1}{4}$–$\frac{1}{2}$ inch long. There was great variation among individual plants in this regard.

Viable caryopses were apparently produced in these experiments only by slender grama, which did so on all treatments. Its seedlings often appeared in the pots in late summer and autumn. It is not known whether the failure was due to lack of pollination in these cross-pollinated species under greenhouse conditions, or whether physiological causes were involved.

Number of roots

Plants of each species for each treatment were washed out of the pots in August and the numbers of crown roots counted (fig. 6). The numbers were higher in five of the species on the shorter than on the 16-hour photoperiod, although not significantly so except in side-oats and blue grama. In black grama the numbers of roots on plants on 8- and 16-hour photoperiods were approximately equal. The value of the ratio, number of elongated stems/number of roots, was inversely correlated with length of photoperiod for all species except blue grama (fig. 6). This is owing chiefly to the strong inverse correlation between number of stems and length of photoperiod. These results indicate that the greatest numbers of root primordia were initiated per tiller under long photoperiods, a condition probably correlated in turn with the greater number of basal nodes and internodes per tiller which matured before these tillers elongated.

Top and root weights

Dry weights of tops (less crowns) were determined for all plants after clipping and showed tendencies in all experiments comparable with those in figure 6, where data are given for weights of clipped tops (solid bars) and for the combined weights of clipped tops, crowns, and roots (solid bars plus hatching). There were positive correlations between dry weights and length of photoperiod, except in blue and black grama. This is a result generally found in short-day plants. Average top weights of slender and side-oats grama plants harvested in December, previously unclipped and remaining on the same photoperiod since germination in April, were approximately double those of the same species and under similar treatment but harvested in August.

Top-weight/root-weight ratios showed no consistent tendency (fig. 6) among treatments but emphasized the high proportion of top weight (clipped top weight plus crowns) in the total weight, a result which would not be expected if these plants were grown in native environments.

Discussion

The results show definitely that certain strains of some species of *Bouteloua* are

sensitive to photoperiod. Within the limits of the experiments, side-oats and slender grama were most so. These species belong to a different subgenus (Atheropogon) from the others. Slender grama is a "typical" short-day plant in all its responses, practically all individuals failing to flower on photoperiods above the critical one, which lies between 14 and 14½ hours. They flowered profusely on photoperiods down to 8 hours, under variable conditions of light intensity. This is in accord with the probable evolutionary history of the species. Its present distribution indicates origin in low latitudes, since it now occurs only in latitudes below 35°, where maximum photoperiods do not exceed its apparent critical one. Northward extension of the strain may well be limited in part by this adaptation to short photoperiods. Other species in the same subgenus, except side-oats grama, are found only in low latitudes.

Side-oats grama ranges to higher latitudes in both South and North America (to 50° N. for the latter). The results here reported show that the strain from Arizona consists chiefly of short-day plants with a critical photoperiod between 14 and 14½ hours. It should be remembered, however, that a few plants of both side-oats and slender grama were able to flower, although delayed in so doing, on 16-hour photoperiod. These individuals, especially side-oats grama, may be of considerable significance from the standpoint of varietal adaptation, even though of little importance in adjustment to their Arizona environment. Additional studies (**19**), not yet reported in full, show that investigated strains of side-oats grama from northern United States consist chiefly of individuals which may be classed as long-day plants, while those from southern United States are mostly short-day (or possibly intermediate). Since the species has undoubtedly spread from south to north in the United States, these results suggest that the northern populations of predominantly long-day plants may have been evolved during this extension of range from populations which were primarily short-day in response in the lower latitudes. If so, a thorough genetic analysis (with respect to photoperiodic response) of a population such as that from southern Arizona, which includes a few individuals not showing the "typical" response, would be of interest. It might throw light upon the possible evolutionary mechanism which led to the present nice adjustment of response to the seasonal range in length of photoperiod as the area was extended from south to north. For example, it seems probable that some of the individuals showing less typical photoperiodic responses, such as those from Arizona able to flower on 16-hour photoperiod, would have been the ones perpetuated through natural selection in the northward migration; although no answer is suggested as to why the northern strains are now chiefly long-day rather than indeterminate. This variability in response to photoperiod in local populations should be taken into consideration in selection and breeding programs, either in interpreting "earliness" in flowering of certain individuals or in cross-breeding of desirable strains from different latitudes.

The investigated members of the subgenus Chondrosium (Rothrock's, hairy, blue, and black grama), probably also originating in the low latitudes, to which many (including black and Rothrock's) are still restricted, apparently are less sensitive to photoperiod as far as flower-

ing responses are concerned. They do exhibit many vegetative responses typical of short-day plants. The strains of the three species from Arizona (black, Rothrock's, and hairy grama) fell into the less typical group of short-day plants (3) which are delayed in flowering on long photoperiods as compared with short ones. If they have a well-defined critical photoperiod, it is longer than any provided in these experiments. Photoperiodic conditions and adaptations have probably been of less importance in the evolutionary development of these species. The possible delay in initiation of reproductive activity in these strains, as well as a midsummer inhibition of flowering in slender and side-oats grama when they all renew vegetative growth under the long photoperiods of the well-defined summer rainy season in southwestern United States, probably leads to greater forage production than if they were completely indeterminate and were to initiate flowering earlier in the season.

The blue grama strain from Montana most closely approaches the indeterminate category, although it showed long-day tendencies. These are in accord with the photoperiodic conditions encountered during the Montana growing season, although the species probably originated in lower latitudes. LAVIN'S studies (15) suggest that in its spread to the north it has become adapted to the increasing range of photoperiods encountered in a somewhat different fashion from that of side-oats grama.

The results indicate that length of photoperiod is undoubtedly a factor of considerable importance in affecting the type of vegetative growth produced at different seasons of the year by all the species investigated; they suggest strongly that knowledge of such responses to photoperiod, as well as flowering responses, would be helpful in guiding efforts to obtain desirable strains of these species through selection and breeding, either for forage production or for use in reseeding overgrazed or denuded areas. Such knowledge certainly would aid in interpreting the variations in growth habit which have been described (10, 14, 21, 24) when strains from different latitudes are grown side by side.

Summary

1. Lots of 100 plants each of side-oats grama (*Bouteloua curtipendula*), slender grama (*B. filiformis*), Rothrock's grama (*B. rothrockii*), hairy grama (*B. hirsuta*), black grama (*B. eriopoda*), and blue grama (*B. gracilis*) were grown continuously for $4\frac{1}{2}$–5 months after germination on April 6 on photoperiods of 16, 12, and 8 hours in the greenhouse. Reciprocal transfers were then made among treatments, both with and without simultaneous clipping of the tops, and the plants were allowed to grow for another 4–6 months. Southern Arizona, except for blue grama from Montana, was the seed source of all species.

2. Growth and flowering responses showed that these strains of slender and side-oats grama, which belong to the same subgenus (Atheropogon), are "typical" short-day plants, most individuals failing to flower on a 16-hour photoperiod. A critical-photoperiod experiment showed this value to lie between 14 and $14\frac{1}{2}$ hours.

3. The other four species, belonging to the subgenus Chondrosium, made less decisive flowering responses. The species with strains from Arizona were considerably delayed in flowering on the longest photoperiod, and these strains

should probably be regarded as short-day plants. Blue grama from Montana was more or less indeterminate, with some long-day tendencies in flowering behavior.

4. The five species with strains from Arizona exhibited more or less typical short-day vegetative behavior. The total numbers of tillers, of tillers bearing inflorescences, and of crown roots were inversely correlated with length of photoperiod to a greater or less degree in most species; while average maximum height, average dry weights of roots and tops, and vigor of individual inflorescences were correlated positively with length of photoperiod.

5. The genus is definitely sensitive to photoperiod, and its effect should be considered in interpreting growth habits of the species in nature and in selection and breeding programs designed to develop strains more desirable for forage production or for use in artificial revegetation of overgrazed or denuded areas.

It is a pleasure to acknowledge with appreciation the assistance of N. J. SCULLY, JOHN DIBBERN, and FRED LAVIN in the experimentation.

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EFFECTS OF GROWTH-REGULATING SUBSTANCES ON PROPAGATION OF GOLDENROD

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Material and methods

Solidago leavenworthii, one of the plants whose rubber-producing potentialities are being extensively investigated at the present time, is available in a considerable number of selected clones propagated mainly from cuttings. Various clones differ considerably in the rubber-producing capacities of their leaves, resistance to disease, degree to which they lose their lower leaves in late season, and other characters that affect the yield of rubber.

Although growth substances have been found effective in the rooting of cuttings of many kinds of plants, apparently they have not been tested with goldenrod, for no reference to their use with this plant is found in a recent review of the subject (2).

The material for this investigation was obtained through the Rubber Investigation Project from plantings at Savannah, Georgia. It consisted of plants from four clones—3 S-4, 3 S-40, 3 S-79, and 3 S-91—that had been selected for their high yield of rubber. The plants had been started from cuttings planted in Savannah on March 30, 1942, in Blanton loamy fine sand. In December the plants were dug and divided into stolons and stems and shipped the same day to Beltsville, Maryland, where the material was prepared for treatment. The stolons were firm and succulent, but the stems were old and woody, had lost their leaves, and become brown in patches. These old stems were used to compare their rooting abilities and to study the effects of growth regulators on such rooting. Other shipments of stolons were received until late in March, and cuttings of stolons were made from December until April.

Cuttings from young stems were later obtained from plants grown in the greenhouse from the stolon cuttings. This material was available about April 1, 1943, and cuttings of it were made from that time until June, 1943.

The growth regulators used were indolebutyric acid, α-naphthalene acetamide, β-naphthoxyacetic acid, α-naphthaleneacetic acid, and a mixture of equal parts of them. The carriers for these substances were either powdered talc or water.

The concentrations of the growth regulators in the powder were 50, 200, and 1000 p.p.m. In making the talc dust, a known amount of the growth regulator was first dissolved in a few cubic centimeters of 95% ethyl alcohol and added to a measured quantity of talc to form a paste. This mixture was dried in an electric oven at about 60°–70° C. and then reduced to a fine powder (2). The lower one-third of the cuttings was dipped in the dust and any excess shaken off. In water the concentrations were 10, 25, 50, and 100 p.p.m., secured by adding a solution of the growth substance in 95% alcohol to the required amount of water. The amount of alcohol used was such that it was less than 0.1% in the final solution. The lower one-third of each cutting was soaked in the solution for 3 or 18 hours.

The stem cuttings were 6 and 3 inches long and the stolons 2 inches and ½ inch long. Stem cuttings were further classified according to diameter as large, medium, and small and according to position on the stem as base, middle, and tip. The stems from which cuttings were made were also classified according to

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their condition and apparent age into young, mature, and over-mature. The over-mature stems had lost their leaves and some of the outer tissues were apparently beginning to die. Stems of both of the other lots were still leafy; those designated as young were 1–2 feet tall and the mature ones 2–5 feet. In most cases forty cuttings were used for each treatment.

After treatment, the cuttings were placed upright in the substrate, basal end down, with about two-thirds below the surface. They were planted at about 1-inch intervals in rows 4 inches apart. In one experiment in which ½-inch stolon cuttings were used, the cuttings were placed horizontally ½ inch below the surface.

Two kinds of substrate were used. One was a sandy, river-bottom type of soil and the other a potting mixture of sand, muck, and soil. The pH of the former was 5.6 and of the latter, 6.7. Between January and April, temperatures of the substrate ranged 75°–85° F. and during May and June, 80°–95° F. The greenhouse was given a light coat of whitewash on the glass about May 15, reducing the light intensity about 25%. When sand was used it was saturated with water at the start of the experiment but received no further watering until after rooting had begun. When soil was used, it was given a thorough soaking at the start of the experiment and light sprinklings every day. The soil, except immediately after sprinkling, was drier than the sand.

Results

Stolons

In January, 1942, stolon cuttings were grown both in sand and in soil to determine whether both types of media would be suitable for use in later experiments. Both media proved satisfactory, but if cuttings were allowed to become established (that is, if the root systems were allowed to develop), the roots grew faster and more extensively in the soil. Ten days (in most cases) after they were first placed in the media, the cuttings were examined to determine any differences in time or amount of rooting resulting from applications of growth substances. If the cuttings were left in the media longer, substrate nutrition, aeration, and other factors might have tended to mask the effects of the growth regulators upon rooting. Stolon cuttings produced the greatest number and amount of roots when the substrate temperature was 70°–80° F., in contrast to lower temperatures. The 3-inch cuttings placed upright in the substrate produced more roots and tops than did the ½-inch ones placed horizontally below the soil surface. Of the latter, more than 50% failed to grow, and the remainder did not grow vigorously (fig. 1). Rotting was much more pronounced in the small underground pieces.

It was apparent from preliminary trials that water was not as satisfactory a carrier for the growth substance as talc dust in the rooting of stolon cuttings. As a result it was used in only a few trials. Indolebutyric acid, and a mixture of indolebutyric, naphthaleneacetic, naphthoxyacetic acids, and naphthalene acetamide were used at 10 and 50 p.p.m. Top growth of cuttings soaked for 18 hours in an indolebutyric-acid solution at 50 p.p.m., or in a mixture at 50 p.p.m., was inhibited. Soaking in indolebutyric acid at 10 p.p.m. for 18 hours produced slightly more roots than controls which had been soaked 18 hours in water only (fig. 2). Soaking in an indolebutyric-acid solution at 50 p.p.m. for 3 hours produced many more roots than the controls,

whether the substrate was at 60°, 70°, or 80° F. Cuttings soaked in indolebutyric acid at 100 p.p.m., or merely dipped at 1000 p.p.m., showed marked top inhibition (fig. 3).

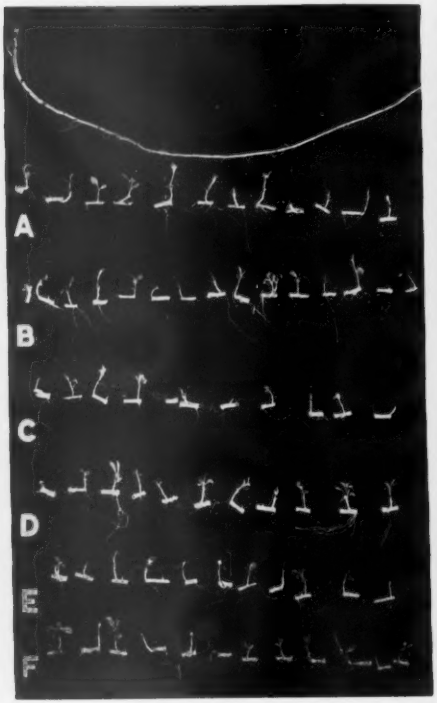

FIG. 1.—Effect of growth substance in talc on ½-inch stolon cuttings of goldenrod. *A*, untreated; *B*, talc control; *C*, ibc, 1000 p.p.m.; *D*, ibc, 200 p.p.m.; *E*, ibc, 50 p.p.m.; *F*, mixture of ibc, nac, nad, noac, total of 200 p.p.m. At top, representative stolon before division.

Stolon cuttings dipped in growth substance dispersed in talc produced more numerous and vigorous roots than did those without treatment. Application of all the growth substances except naphthoxyacetic acid resulted in increased rooting, at least at certain concentrations. Cuttings dipped in dusts containing indolebutyric acid developed the most vigorous root system, as to both number and volume. Indolebutyric acid was most effective at 200 and 1000 p.p.m. (fig. 4, table 1). Roots from cuttings

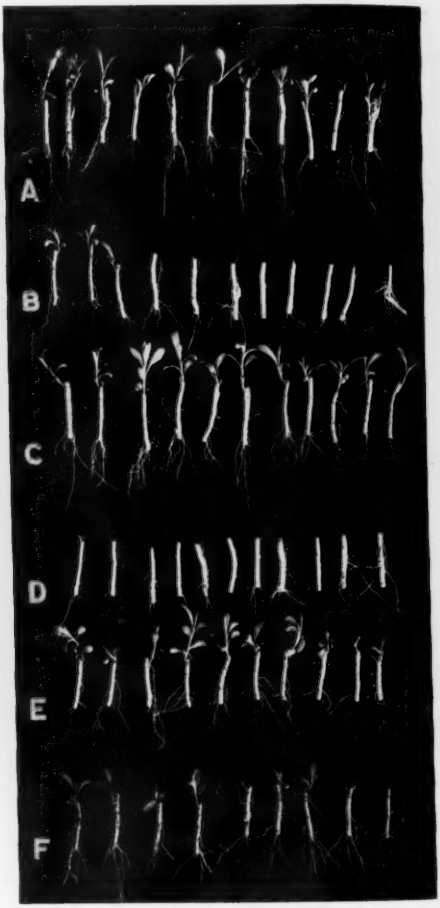

FIG. 2.—Stolon cuttings of goldenrod soaked in solutions of growth regulators for 18 hours. *A*, controls, water only; *B*, ibc, 50 p.p.m.; *C*, ibc, 10 p.p.m.; *D*, mixture of ibc, nac, nad, and noac, total of 50 p.p.m.; *E*, mixture as in *D*, total of 10 p.p.m.; *F*, nac, 10 p.p.m.

treated with indolebutyric were very slender and long in contrast to the shorter and more sturdy roots of the controls.

Cuttings treated with naphthaleneacetic acid at 200 p.p.m. developed a

root system comparable in appearance with those from treatment with indolebutyric at 200 and 1000 p.p.m. Not as many cuttings were heavily rooted as when treated with indolebutyric. Treatment with naphthaleneacetic acid at 1000 p.p.m. induced some inhibition in top growth. Cuttings treated with naphthalene acetamide developed shorter and thicker roots than those treated with indolebutyric or naphthaleneacetic acid, and there were not as many heavily rooted cuttings, although more roots were formed than by the controls. Top growth and root production of material treated with naphthoxyacetic at 200 and 1000 p.p.m. were inhibited. At 50 p.p.m. fewer roots were produced than in the controls.

Mixtures of several growth substances have been shown to be more effective for root formation in cuttings than equivalent concentrations of the individual substance (1). Cuttings of goldenrod treated with a mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic and indolebutyric acids at a total concentration of 50 or 200 p.p.m. produced more roots than the controls, but at 1000 p.p.m. top growth was considerably less. The mixture was not as effective in these tests as was indolebutyric acid.

Stems

As in the case of stolon cuttings, sand and soil were both suitable substrates for rooting. Growth after rooting had begun was more extensive in soil than in sand.

The length of the cutting taken from young and mature stems was not so important a factor as in the rooting of old, over-mature stem cuttings. Three-inch and 6-inch cuttings of young and mature stems rooted readily and profusely, regardless of treatment or diameter size. In the old, over-mature stems, 6-inch cuttings produced more roots than 3-inch ones. However, only about 5% of the cuttings from the over-mature stems rooted; most of them were of larger diameter and taken from the base of the stem.

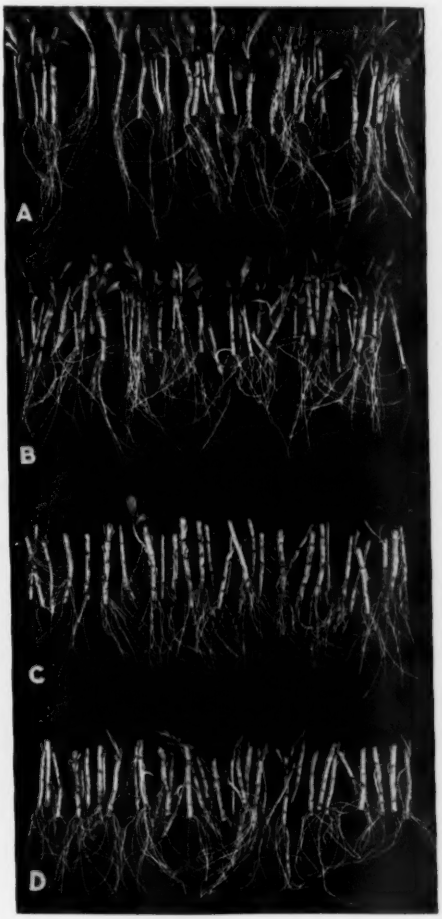

Fig. 3.—Stolon cuttings of goldenrod soaked in solutions of ibc for 3 hours. *A*, water only; *B*, 10 p.p.m.; *C*, 100 p.p.m.; *D*, 1000 p.p.m.

Roots were produced by a much greater percentage of cuttings from stems that still had their leaves than by those

from old, over-mature stems without leaves. A greater number and quantity of roots were found on the cuttings from the young and mature stems. Cuttings from young stems 1–2 feet tall produced more roots than those from mature stems 2–5 feet tall, but the difference was not great.

The region of the stem from which cuttings were made was very important in rooting (table 2, figs. 5, 6). Cuttings from the region nearest the tip in the young stems produced more roots than did those from the middle of the stem or from the base, and cuttings from the middle region produced more roots than did those from the base. The same results were obtained with mature stems 2–5 feet tall. In the over-mature stems in which the leaves had died, the re-

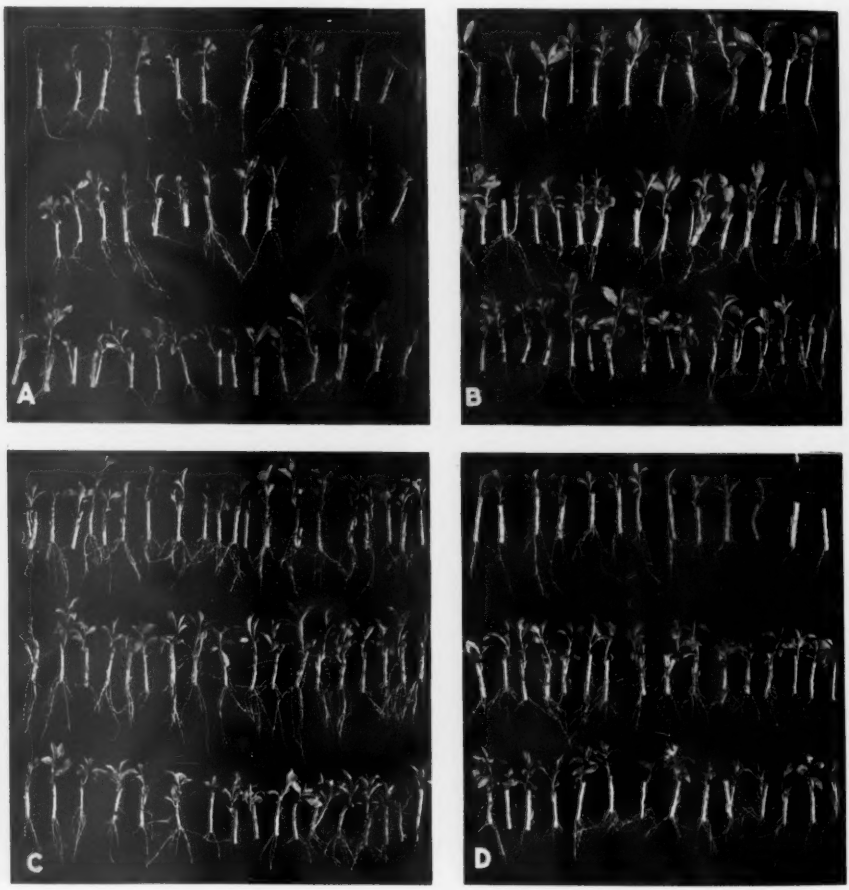

FIG. 4.—Rooting response of stolon cuttings of goldenrod to growth regulators dispersed in talc powder. Top row in each group 1000 p.p.m., middle row 200 p.p.m., bottom row 50 p.p.m. *A*, naphthaleneacetic acid (nac); *B*, naphthalene acetamide (nad); *C*, indolebutyric acid (ibc); *D*, mixture of ibc, nac, nad, and naphthoxyacetic acid (noac), total of 50 p.p.m.

TABLE 1

RESPONSE OF STOLONS OF GOLDENROD CUT 3 INCHES LONG TO GROWTH SUBSTANCES

Lot	Time of application	Treatment: Preparation	Treatment: Concentration (p.p.m.)	Time in solution (hours)	Percentage cuttings showing various degrees of rooting: None	Few	Medium	Heavy	Remarks
		I. Rooted in sand at 65°–75°							
A	Jan.	Water: Control	.....	18	10	90	0	0	Poor rooting but good top growth
B	"	Ibc*	50	18	20	80	0	0	Marked inhibition of tops
C	"	Ibc	10	18	0	40	60	0	Marked improvement over controls
D	"	Mixture of ibc, nac, nad, noac	50	18	40	60	0	0	Top inhibition
E	"	"	10	18	20	80	0	0	Comparable with controls
		II. Rooted in soil at 65°–75°							
A	Jan.	Talc dust: Control	.....	.....	10	70	20	0	.....
B	"	Nac	1000	.....	0	100	0	0	Some top inhibition
C	"	Nac	200	.....	0	20	60	20	.....
D	"	Nac	50	.....	0	70	30	0	.....
E	"	Nad	1000	.....	0	100	0	0	.....
F	"	Nad	200	.....	0	10	60	30	.....
G	"	Nad	50	.....	0	60	40	0	.....
H	"	Noac	1000	.....	70	30	0	0	Too concentrated
I	"	Noac	200	.....	20	70	10	0	.....
J	"	Noac	50	.....	0	100	0	0	.....
K	"	Mixture	1000	.....	20	80	0	0	.....
L	"	Mixture	200	.....	0	0	80	20	.....
M	"	Mixture	50	.....	0	0	80	20	.....
N	"	Ibc	1000	.....	0	0	40	60	.....
O	"	Ibc	200	.....	0	0	60	40	.....
P	"	Ibc	50	.....	10	70	20	0	.....
		III. Rooted in sand at various temperatures							
A. (60° F.)	Feb.	Water: Control	.....	3	60	40	0	0	.....
B. (70° F.)	"	Control	.....	3	30	50	20	0	.....
C. (80° F.)	"	Control	.....	3	30	20	50	0	.....
D. (60° F.)	"	Ibc	50	3	5	45	50	0	.....
E. (70° F.)	"	Ibc	50	3	5	10	55	30	.....
F. (80° F.)	"	Ibc	50	3	5	5	70	20	.....

* Ibc, indolebutyric acid; nac, naphthaleneacetic acid; nad, naphthalene acetamide; noac, naphthoxyacetic acid.

TABLE 2

RESPONSE OF STEMS OF GOLDENROD TO GROWTH SUBSTANCES

Lot	Time of application	Treatment: Preparation	Treatment: Concentration (p.p.m.)	Time in solution (hours)	Percentage cuttings showing various degrees of rooting: None	Few	Medium	Heavy
	I. Young stems about 3 inches tall cut at ground level and rooted in sand at 80°–85°							
A.	Feb.	Water: Control	.....	3	20	46	26	8
B.	"	Ibc*	100	3	20	8	33	39
C.	"	Ibc	50	3	35	35	10	20
D.	"	Ibc	25	3	37	32	18	13
E.	"	Nac	100	3	13	20	20	47
F.	"	Nac	50	3	6	39	6	49
G.	"	Nac	25	3	28	6	40	26
H.	"	Noac	100	3	.....	.....	.....	.....
I.	"	Noac	50	3	29	0	23	48
J.	"	Noac	25	3	48	6	0	46
	II. Young stems about 1 foot tall cut into 3-inch lengths and rooted in sand at 80°–85°							
A. Base	Feb.	Water: Control	.....	.....	0	44	56	0
Middle	"	Control	.....	.....	0	55	45	0
Tip	"	Control	.....	.....	0	33	58	9
B. Base	"	Ibc	25	3	0	61	39	0
Middle	"	Ibc	25	3	0	25	50	25
Tip	"	Ibc	25	3	0	12	33	55
C. Base	"	Ibc	50	3	0	56	44	0
Middle	"	Ibc	50	3	0	29.5	70.5	0
Tip	"	Ibc	50	3	0	22	26	52
	III. Young stems 1 foot tall cut into two pieces in sand at 80° with no shade							
A. Base	April	Talc dust: Control	.....	.....	0	0	92	8
Tip	"	Control	.....	.....	0	0	40	60
B. Base	"	Nad	50	.....	0	0	72	18
Tip	"	Nad	50	.....	0	0	56	44
C. Base	"	Mixture	50	.....	0	0	80	20
Tip	"	Mixture	50	.....	0	0	40	60
D. Base	"	Nac	50	.....	0	0	84	16
Tip	"	Nac	50	.....	0	0	44	56

* Ibc, indolebutyric acid; nac, naphthaleneacetic acid; noac, naphthoxyacetic acid; nad, naphthalene acetamide.

TABLE 2—*Continued*

Lot	Time of application	Treatment: Preparation	Treatment: Concentration (p.p.m.)	Time in solution (hours)	Percentage cuttings showing various degrees of rooting: None	Few	Medium	Heavy
	IV. Young stems approximately 3 feet tall and about ¼ inch in diameter at base, cut into 6-inch lengths and rooted in sand at 90° with shade							
		Talc dust:						
A. Base	April	Control	.....	.....	0	45	50	0
Middle	"	"	.....	.....	0	35	50	15
Tip	"	"	.....	.....	0	20	50	30
B. Base	"	Ibc	50	.....	0	44	56	0
Middle	"	"	50	.....	0	9.5	70	20.5
Tip	"	"	50	.....	0	12	52	36
C. Base	"	Mixture	50	.....	0	16	36	48
Middle	"	"	50	.....	0	8.1	46.6	45.3
Tip	"	"	50	.....	0	0	52	48
D. Base	"	Nac	50	.....	0	72	28	0
Middle	"	"	50	.....	0	49	33	18
Tip	"	"	50	.....	0	0	36	64
	V. Young stems 1 foot tall, cut into 4-inch pieces and grown in sand at 80°							
		Water:						
A.	April	Control	.....	3	0	40	52	8
B.	"	Nad	25	3	0	34	44	22
C.	"	Nad	50	3	0	24	48	28
D.	"	Nac	25	3	0	26	60	14
E.	"	Nac	50	3	0	36	52	12
F.	"	Noac	25	3	0	50	42	8
G.	"	Noac	50	3	0	48	20	32
H.	"	Ibc	25	3	0	26	48	26
I.	"	Ibc	50	3	0	28	22	50
	VI. Mature stems 4–5 feet tall, cut into 6-inch lengths examined after 16 days							
		Water:						
A. Base	May 29	Control	.....	3	9.5	40	51.5	0
Middle	"	"	.....	3	12	34	40	14
Tip	"	"	.....	3	30	55	15	0
B. Base	"	Ibc	50	3	0	0	46	54
Middle	"	"	50	3	0	42	12	46
Tip	"	"	50	3	6	18	6	70

verse was the case; the cuttings from the basal region produced more roots than those from the middle or top region. were dipped in talc. Three hours was sufficient time for soaking the young and mature stem cuttings in a solution.

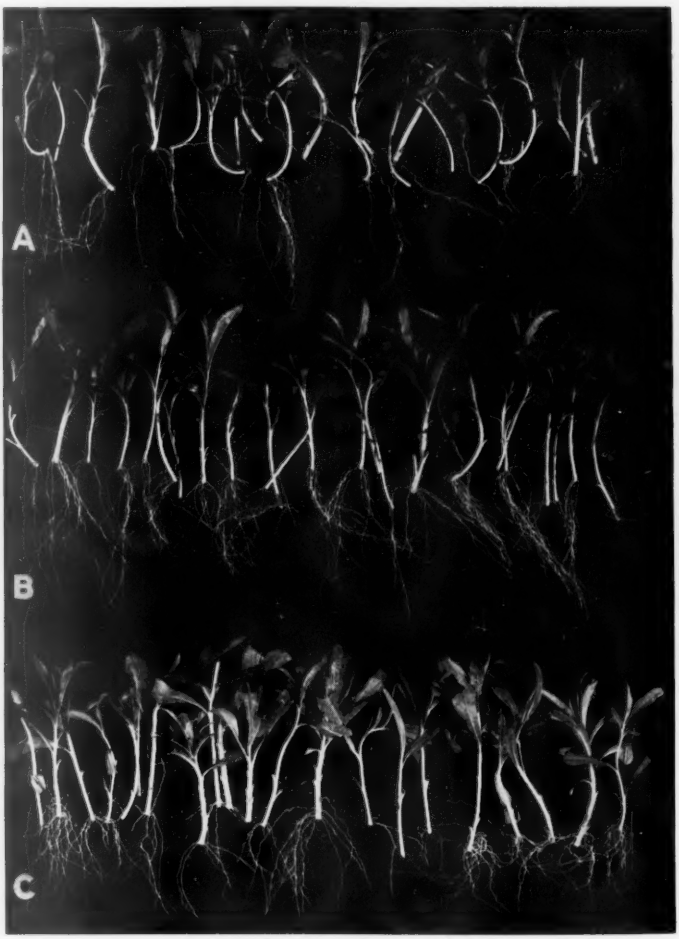

FIG. 5.—Cuttings of young goldenrod stems soaked in solutions of ibc for 3 hours. *A*, water only; *B*, 25 p.p.m.; *C*, 100 p.p.m.

Data for the over-mature cuttings were not included.

In contrast to stolon cuttings, stem cuttings produced more roots when they were soaked in a solution than when they

In a number of experiments on young stems, some of which are recorded in table 2, it was found that, although the response of young stem cuttings to growth substances varied with each ex-

periment, cuttings soaked in indolebutyric and naphthaleneacetic acid consistently produced more roots than the controls.

From over-mature stems a greater number of cuttings rooted when soaked in naphthaleneacetic or indolebutyric

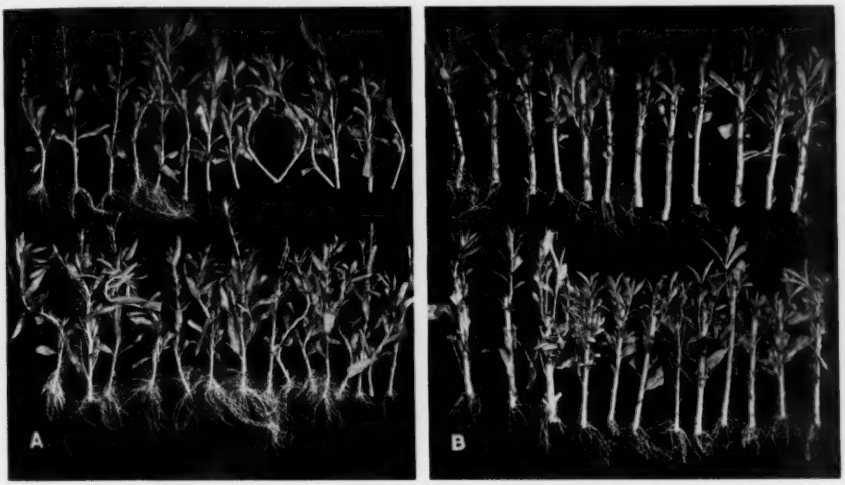

FIG. 6.—Rooting of tip and basal sections of mature goldenrod stems. *A*, tip region: upper row, controls; lower row, soaked in aqueous solution ibc, 50 p.p.m., for 3 hours. *B*, basal region: upper row, controls; lower row, aqueous solution ibc, 50 p.p.m.

acid at 100 p.p.m. for 18 hours than without treatment. A greater amount of rooting occurred on the treated cuttings. Although data are not here provided, only a small percentage of over-mature stems rooted, and they required longer for rooting than did young or mature stems.

Talc dust was a satisfactory carrier for the growth substances but less so than water. Cuttings treated with any of the growth substances at 50 p.p.m. in talc produced more roots than did the controls (table 2).

Summary

STOLONS

1. Stolon cuttings of goldenrod soaked in aqueous solutions of several growth substances at concentrations of 10 to 100 p.p.m. for 18 hours were inhibited in top growth. Some of the cuttings rotted following this long treatment. Soaking for 3 hours in indolebutyric-acid solution at 10 or 50 p.p m. gave some increase in rooting over the controls.

2. Cuttings dusted with indolebutyric, naphthaleneacetic, naphthoxyacetic acids, or naphthalene acetamide, or a mixture of these four, dispersed in powdered talc produced more roots than did those without treatment, except when the highest concentrations were used.

3. When the temperature of the sub-

strate was 70°–80° F., the greatest number and quantity of roots were formed.

4. Regardless of treatment, stolons cut into 3-inch pieces produced many roots and heavy top growth. Those cut into ½-inch pieces produced few roots and only 50% of the pieces rooted; top growth was slight.

STEMS

5. Cuttings from young stems produced a more vigorous root and top system than did cuttings from more mature stems. Cuttings from the tips of both young and mature stems rooted more abundantly than did cuttings from the base of the stems.

6. Only a small percentage of cuttings from old, over-mature, leafless stems formed roots, and these were not numerous.

7. Cuttings soaked in aqueous solutions of indolebutyric acid at 25, 50, or 100 p.p.m. for 3 hours produced more roots than did the controls.

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BELTSVILLE, MARYLAND

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STRUCTURE AND DEVELOPMENT OF SYMPHYOGYNA BRASILIENSIS

ARTHUR W. HAUPT

Introduction

According to SCHIFFNER (10), *Symphyogyna* includes 27 species. A few of these are found in tropical parts of the Northern Hemisphere, but most of them occur only south of the Equator, where they range from tropical to antarctic regions. STEPHANI (11) recognized a greater number of species than SCHIFFNER, fifty-six in all, to which EVANS (2, 3) has added several new ones from America. Although the classification of the anacrogynous Jungermanniales is a matter of considerable difference of opinion, especially regarding the number and delimitation of the families, there is no doubt as to the closeness of relationship between *Symphyogyna* and *Pallavicinia*. All authorities place them in the same family, which has been variously designated as the Dilaenaceae, Pallaviciniaceae, Blyttiaceae, or Leptotheceae.

Symphyogyna brasiliensis Nees is widely distributed in tropical America, ranging from Mexico to Bolivia and Brazil. Material for the present study was collected by the writer in Costa Rica during July and August of 1940. This species, often associated with *S. brogniartii* Mont., was found in several parts of the mountains lying both to the north and south of the city of San José. Specific localities are given elsewhere (7). The most vigorous plants were found in densely wooded ravines, growing in shade on rich moist humus.

Most of the material was fixed in Randolph's modification of Navashin's fluid, which was allowed to act for 2–3 days. Some material was also fixed in a 3.5% solution of glacial acetic acid to which had been added 0.5 gm. of chromic-acid crystals to each 100 cc. of solution. This was allowed to act for only 24 hours. Both fluids gave thoroughly satisfactory results. After washing for about 12 hours or more, the material was transferred to a 5% solution of formalin. Several months later it was prepared for imbedding in paraffin by the ethyl alcohol-xylol technique, sectioned at 8 μ, and stained in iron-alum haematoxylin, followed by either erythrosin or orange G.

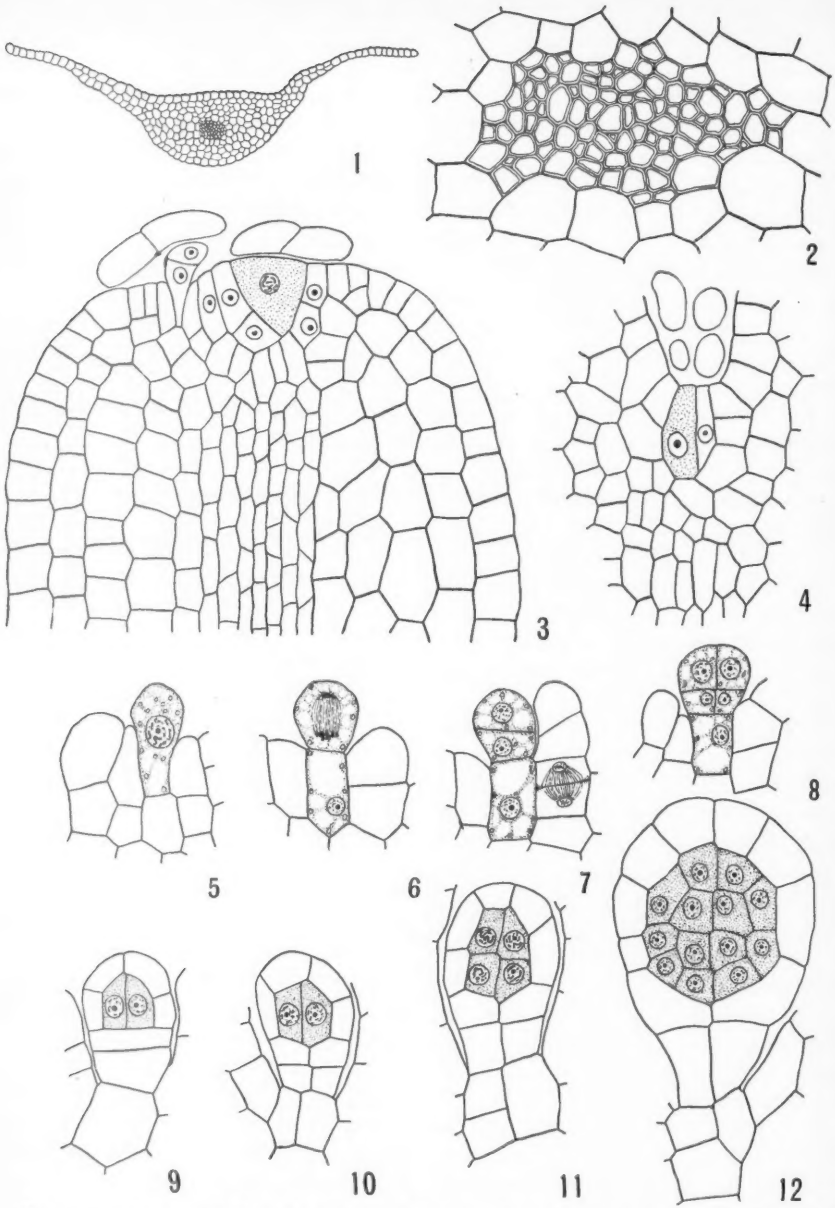
Previous morphological work on *Symphyogyna* has been done by LEITGEB (8), who studied *S. subsimplex*, *S. rhizoloba*, and *S. sinuata*, and by MCCORMICK (9), who studied *S. aspera*.

Observations

THALLUS

S. brasiliensis is one of a group of species having a ribbon-like prostrate thallus bearing rhizoids on its lower surface. Except when young, however, the tips of the thallus tend to become ascending and, not being in contact with the substratum, fail to produce rhizoids. This is especially true of plants growing in crowded mats. The thalli are light green and often more or less tinged with reddish purple. Plants growing in open situations are more likely to show this reddish pigmentation than those living in moist shady places.

The thallus consists of a distinct midrib and lateral wings, the latter gradually thinning to a single layer of cells (fig. 1). The wings are unistratose for one-half or more of their width. The upper surface of the thallus is nearly flat, the midrib projecting below and bearing long colorless rhizoids where it touches the soil. Ventral scales are lacking. The extreme base of the thallus is without wings; it is stalklike and only 2–3 mm. long. The entire thallus is generally 10–20 mm. long, but under optimum conditions it may reach a length of 35–45 mm. or more.



FIGS. 1–12.—Figs. 1–4, thallus: Fig. 1, transverse section. Fig. 2, central strand of conducting cells. Fig. 3, median vertical section of apex showing apical cell and young archegonium. Fig. 4, horizontal section of same. Figs. 5–12, development of antheridium: Fig. 5, antheridium initial. Fig. 6, formation of basal cell and division of outer cell. Fig. 7, formation of primary antheridial cell and primary stalk cell. Fig. 8, appearance of vertical walls. Figs. 9, 10, appearance of periclinal walls, differentiating jacket, and spermatogenous cells. Figs. 11, 12, later stages. Fig. 1, ×25; figs. 2–4, ×350; others ×500.

The male plants average 2–3 mm. in width but are occasionally up to 4 mm. wide. The female plants are larger, averaging 4–5 mm., or sometimes as much as 6 mm., in width. The margins of the thallus are slightly undulate in the vertical plane and, in the material studied, are entirely without teeth or lobes. Most of the plants are unbranched, but many show a single dichotomy; a few plants exhibit two or even three dichotomies. Occasionally plants with ventral adventitious branches are seen, these narrowing to a stalklike base that arises from alongside the midrib.

The midrib is traversed by a central strand of narrow, elongated conducting cells with thick pitted walls and pointed ends (fig. 2). They are differentiated immediately behind the apical cell (fig. 3). The conducting strand, as seen in cross section, comprises about eighty cells. It lies at or slightly below the center of the midrib. In *Pallavicinia*, which has the same kind of conducting cells as *Symphyogyna*, TANSLEY and CHICK (12) have demonstrated, by means of eosin solutions, that they conduct water. In *S. aspera* MCCORMICK (9) has shown that their walls are composed of pectose.

All cells in the younger portion of the thallus (except those forming the conducting strand) contain chloroplasts, while in older parts many starch grains are frequently present in cells below the epidermis. Intracellular fungi inhabit the thallus, but not as a rule in the portion that is free from the substratum.

The apex of the thallus is deeply notched. It contains an apical cell that, in the material studied, is of the cuneate type. As seen in median vertical section, the apical cell is broadly triangular in outline, cutting off segments both above and below (fig. 3). In a horizontal section the apical cell appears rectangular, or nearly so, and cuts off segments to the right and left (fig. 4). Numerous sections cut in this plane were examined and in none was the apical cell pointed at the base, as would be true of a dolabrate apical cell. In a transverse section the apical cell also appears rectangular. Short 2-celled mucilage hairs arise in the growing notch both above and below the apical cell.

Although an apical cell of the dolabrate type is characteristic of the majority of the anacrogynous Jungermanniales, several exceptions have been noted, as in *Pellia*, *Blasia*, and *Moerckia*. In the three species of *Symphyogyna* that he studied, LEITGEB (8) found a dolabrate apical cell. In *S. aspera* MCCORMICK (9) found two types of apical cell, the dolabrate and the cuneate, each characteristic of plants collected in two different places in Mexico. In *Pallavicinia* a dolabrate apical cell has been seen by CAMPBELL and WILLIAMS (1), who investigated three different species, and by HAUPT (4), who studied *P. lyellii*.

SEX ORGANS

Like other species of the genus, *S. brasiliensis* is dioecious, the male plants being more slender than the female. The sex organs, always borne on the main thallus, are dorsal in position, occurring along the midrib. The antheridia are in long crowded groups that often extend almost the entire length of the thallus. They form a band, about 1 mm. wide, in which they are scattered irregularly, although often appearing to be arranged in three to five indefinite rows. The antheridia lying along the center of the midrib point forward, while those along its sides point diagonally outward. They are not sunken in the thallus, but each is inclosed by a scalelike posterior involucre that arches toward the apex of the thallus.

The archegonia occur in isolated groups lying directly upon the midrib. These groups are generally 4–6 mm. apart. The number of archegonia in a group is not so high as in certain other species, such as *S. brogniartii*, but reaches about twelve. The necks of the archegonia lie parallel with the surface of the thallus and point forward. According to EVANS (3), "the female inflorescences [of *S. brasiliensis*] are borne singly or in small numbers, three on one thallus being the highest number observed." The writer has seen many plants with more than three archegonial groups. One plant, unbranched and measuring 42 mm. in length, had ten archegonial groups, equally spaced, extending along its entire length.

Each group of archegonia, slightly raised on a pad, is covered by a flaplike posterior involucre that is open in front but attached to the thallus behind and on the sides. The involucre is partially dissected into two or three segments, each of which may be somewhat laciniate.

ANTHERIDIUM

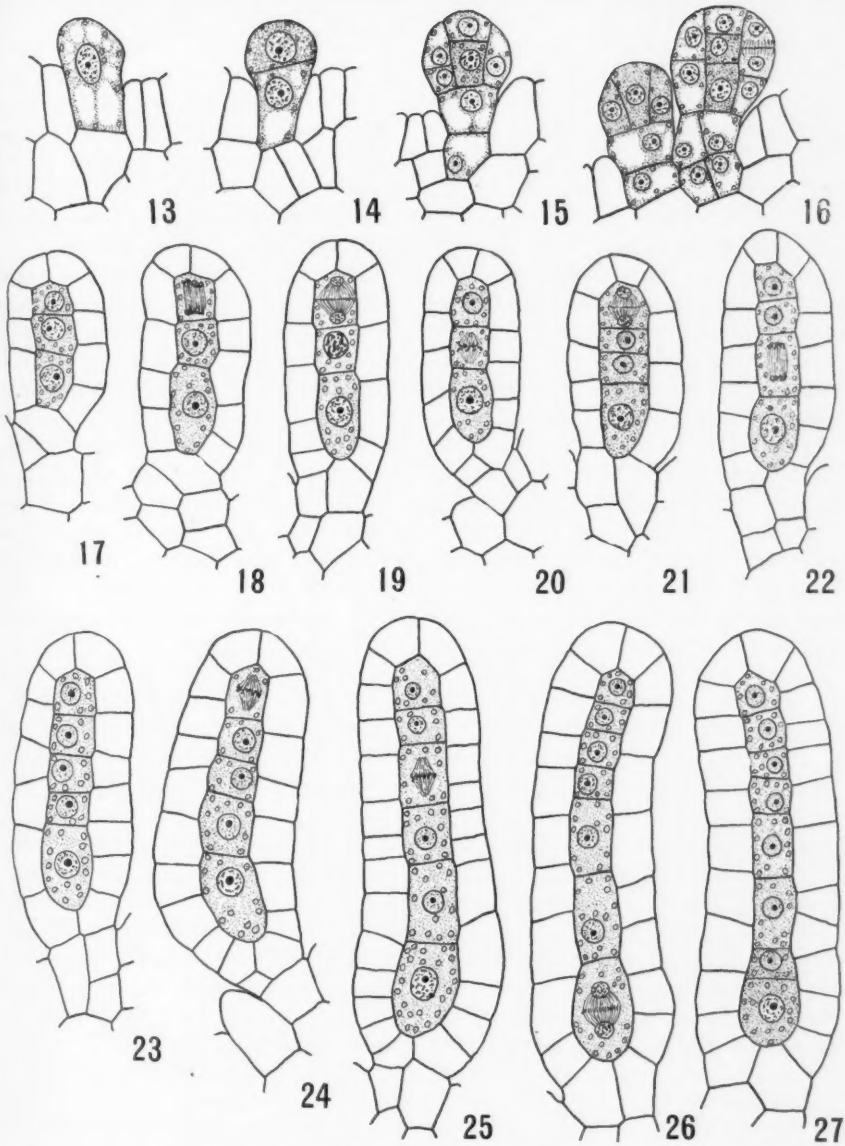
The antheridia arise close to the apical cell and develop in acropetal succession from its immediate dorsal segments, generally from the third or fourth one. The development is similar to that previously described for *Pallavicinia* (4) and *Fossombronia* (5, 6). The initial is papillate and, by a transverse division, gives rise to an outer free cell and a basal imbedded cell (figs. 5, 6). The outer cell undergoes another transverse division into two approximately equal segments, the upper one being the primary antheridial cell and the lower one the primary stalk cell (figs. 6, 7). The next division, a median vertical one in the antheridial cell, is followed by a division in the stalk cell that is usually vertical but may be transverse (fig. 8).

Generally two additional divisions complete the stalk, while a periclinal wall, intersecting the median wall, is now established in each of the two cells derived from the primary antheridial cell. Two additional periclinal walls, coming in at right angles to the first periclinal ones, intersect both these and the median wall. As a result four outer sterile cells are separated from two central spermatogenous cells, as in all the Jungermanniales (figs. 9, 10). The outer cells increase in number by anticlinal divisions and give rise to a single-layered sterile jacket, while the spermatogenous cells multiply by divisions in all three planes (figs. 11, 12). The cells of the sterile jacket at this and subsequent stages contain chloroplasts, but the spermatogenous cells do not. The antheridium soon assumes a spherical form, its stalk remaining very short. Later stages resemble those of other anacrogynous Jungermanniales.

At a very early stage in the development of the antheridium, the involucre appears as an upgrowth of the thallus immediately behind it (fig. 7). It consists of a single layer of cells that soon overarches the antheridium, finally reaching a length of about 7–9 cells.

ARCHEGONIUM

The archegonia originate very close to the apical cell, arising, as MCCORMICK (9) has observed, from approximately the fourth dorsal segment (fig. 3). Their development is not strictly acropetal, however, since young archegonia may continue to arise among the older ones, as in *Pallavicinia* (4). The papillate initial divides transversely to form an outer free cell and a basal imbedded cell (figs. 13, 14). The usual three intersecting verti-



FIGS. 13–27.—Early stages in development of archegonium: Fig. 13, archegonium initial. Fig. 14, first division of initial. Fig. 15, formation of central cell and cover cell. Fig. 16, formation of primary axial cell and primary wall cells (left) and of primary ventral cell and primary neck canal cell (right). Figs. 17–25, formation of neck canal cells. Figs. 26, 27, differentiation of ventral canal cell and egg from primary ventral cell. ×440.

cal walls now appear in the outer cell, cutting off three primary jacket cells from the primary axial cell, while a transverse division occurs in the basal one (fig. 16). The cover cell and central cell are now differentiated from the primary axial one (fig. 15), the central cell soon giving rise to the primary neck canal cell and primary ventral one (fig. 16). The primary neck canal cell divides transversely to form an upper and a lower segment, while—either at this stage or at the one immediately following—the cover cell divides by a vertical wall (fig. 17).

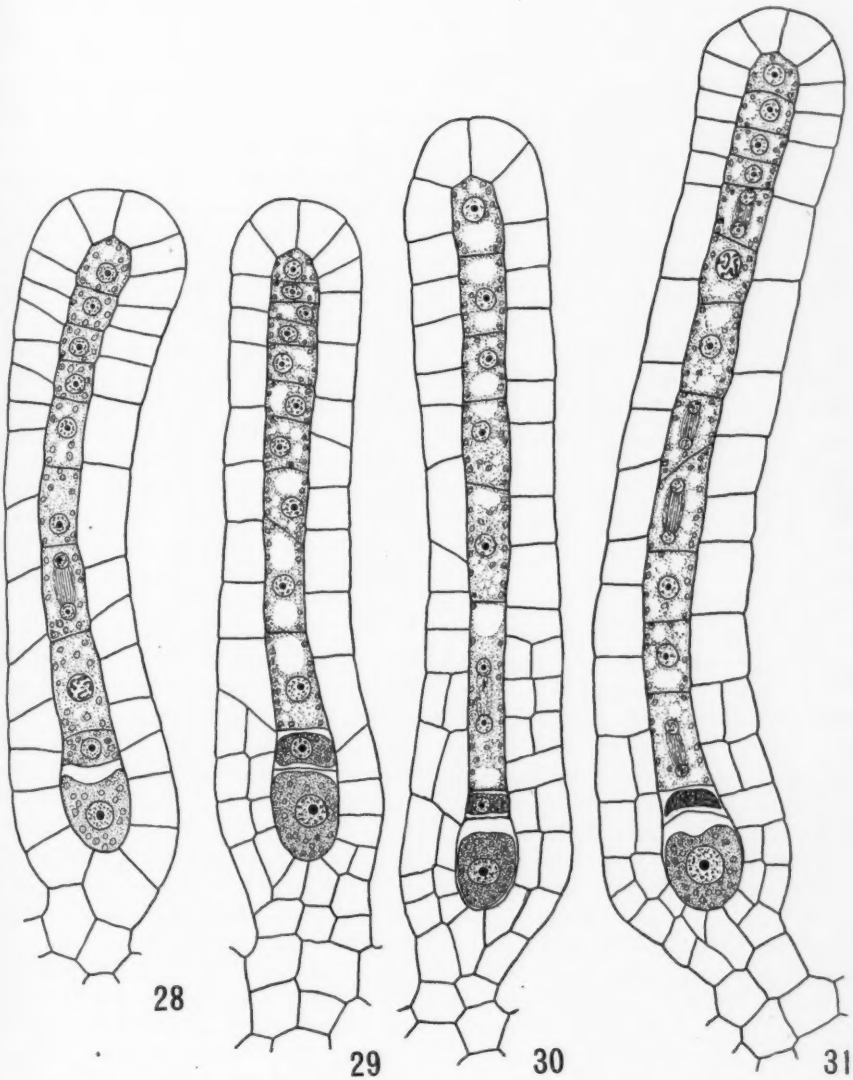
Each of the two cells derived from the primary neck canal cell again divides transversely, the first division occurring either in the upper cell (figs. 18, 19, 22) or in the lower one (figs. 20, 21). Sometimes the division of the second cell is begun before that of the first one is completed (fig. 19). The young archegonium now consists of a primary ventral cell, four neck canal cells, and a single layer of outer cells forming the sterile jacket (fig. 23). As a rule, each of the two uppermost neck canal cells undergoes another transverse division (figs. 24, 25), and then division of the primary ventral cell takes place, differentiating the ventral canal cell and egg (figs. 26, 27). The division of the primary ventral cell may occur somewhat earlier, however, one archegonium having been seen with an egg, a ventral canal cell, and five neck canal cells. In this case only the uppermost of the four original neck canal cells had again divided.

As the protoplast of the egg begins to round off, the neck of the archegonium continues to increase in length and the neck canal cells continue to increase in number (figs. 28–31). At the same time the venter becomes 2-layered. The upper neck canal cells divide more rapidly than the lower ones, and although there is no indication that the divisions occur in strict acropetal sequence, there is a tendency in this direction. The archegonium represented by figure 31 is 350 μ long but has not yet reached its full length, which measurement of slightly older archegonia shows to be approximately 500 μ.

The final divisions of the neck canal cells are unaccompanied by cross walls, so that many or all of them are binucleate. The number of neck canal nuclei may ultimately reach eighteen or twenty. The greatest number of neck canal cells counted by McCormick (9) was thirteen, but she says nothing about any of them being binucleate. The neck becomes curved and frequently twisted, so that it is not always easy to determine from a series of consecutive sections the exact number of neck canal cells and nuclei finally present.

At all developmental stages the venter is slender, only slightly exceeding the neck in diameter. The ventral canal cell is relatively large. It becomes very dense and begins to break down before the neck canal cells show any evidence of disintegration. At all stages the cells of both the axial row and jacket contain numerous plastids. The neck canal cells are surrounded by five rows of neck cells. The involucre begins to develop behind the archegonia as soon as two or three of them have arisen from the immediate dorsal segments of the apical cell. At first it is only one layer of cells thick, but later the basal half becomes 3- or 4-layered. Around and among the archegonia, as noted by Leitgeb (8), are numerous 2-celled, clavate mucilage hairs similar to those found at the apex of the thallus.

In *Pallavicinia lyellii* the writer (4) found that about ten neck canal cells are formed before the ventral canal cell and egg are differentiated, but that addition-



FIGS. 28–31.—Older stages in development of archegonium, showing rounding off of ventral canal cell and egg and formation of additional neck canal cells. ×440.

al neck canal cells may arise later. Some of these are binucleate, the total number of nuclei sometimes reaching eighteen. The venter becomes 2-layered previous to fertilization, about when the ventral canal cell begins to disorganize. In both *Pallavicinia* and *Symphyogyna* the belated division of the ventral cell to form the ventral canal cell and egg and the subsequent increase in the number of neck canal cells may be regarded as primitive features.

SPOROPHYTE

Not enough stages in embryogeny were found to make possible a detailed account. The few stages that were observed indicate that the sequence is essentially the same as in *Symphyogyna rhizoloba*, as presented by LEITGEB (8), and *S. aspera*, as given by MCCORMICK (9).

The first division of the fertilized egg, which is transverse, is followed by a similar division that, according to LEITGEB, occurs in the epibasal segment. Thus a row of three superimposed cells is formed. As a result of the establishment and functioning of a dolabrate apical cell, the embryo soon becomes elongated. The uppermost part is most active in cell division, the middle part in elongation, while the innermost cell seems to divide only a very few times. No evidence has been seen of the formation of a haustorium-like appendage to the foot, such as is present in *Pallavicinia*. In *S. aspera* (9) three wall layers are formed following delimitation of the amphithecium from the endothecium. The cell walls of the outer layer later thicken uniformly without forming semiannular bands, and the two inner layers do not entirely disappear. As is well known, a pseudoperianth is not developed in *Symphyogyna*, but the calyptra becomes thick and fleshy, being many-layered almost to the apex and with a cluster of about twelve unfertilized archegonia at its summit. In *S. brasiliensis* the calyptra becomes 4–5 mm. long and approximately 0.8 mm. in diameter. Normally only one sporophyte develops in each archegonial group.

Formation of the spore mother cells has been thoroughly studied by MCCORMICK (9). The sporogenous tissue, differentiated relatively late, is at first composed of uniform cells. The cells that are to become elaters elongate without further division, while those that ultimately give rise to spore mother cells divide several times. The protoplasts of the latter withdraw from their cell walls, which break down to form a gelatinous matrix. The mother cells assume an irregular amoeboid form, finally becoming prominently 4-lobed. The process of lobing is accompanied by the formation of large vacuoles.

The mature capsule is long-stalked and cylindrical. Its wall is composed of two layers of cells, the cell walls of the outer layer being uniformly thickened. At the apex of the capsule the wall is four or five layers thick, forming a prominent sterile cap. This bears no relation to the elaters. The foot is slender and rounded. It is not sharply differentiated from the seta. The elaters have two spiral bands of thickening and, according to EVANS (3), vary in length from 100 to 800 μ. They fall away in dehiscence. The mature spores are 22–26 μ in diameter, their wall being covered with low ridges running in all directions. The capsule reaches a length of 2.5–3.0 mm. and a diameter of 0.6–0.8 mm. Dehiscence takes place by means of four valves that remain united at the apex.

Summary

1. The thallus of *Symphyogyna brasiliensis* grows by means of a cuneate api-

cal cell. Branching is generally apical but sometimes ventral and adventitious.

2. The thalli are strictly dioecious, the male plants being smaller than the female.

3. The antheridia are crowded and scattered irregularly in a long band lying above the midrib, each inclosed by a posterior involucre.

4. Development of the antheridium follows the general pattern of the anacrogynous Jungermanniales.

5. The archegonia are in isolated dorsal groups of about twelve, each group on a pad and covered by a posterior involucre.

6. In the development of the archegonium, generally six neck canal cells are formed before the ventral canal cell and egg are differentiated.

7. The number of neck canal cells is later increased, often up to twelve or fifteen; since some of these are binucleate, there are often as many as twenty neck canal nuclei.

8. The venter becomes 2-layered before fertilization.

9. The embryogeny follows the sequence of stages previously described for other species.

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ORIGIN AND DEVELOPMENT OF EMBRYOS IN CERTAIN APOGAMOUS FORMS OF DRYOPTERIS[1]

ROBERT E. DUNCAN

The first description of apogamy in *Dryopteris* is that of DE BARY (1) who reported that prothallia of *Aspidium filix-mas* var. *cristatum* form embryos in the same manner as prothallia of *Pteris cretica* var. *albo-lineata*. The second study is that of LANG (7) who listed *Nephrodium propinquum* var. *cristatum* and *N. filix-mas* var. *cristatum* as forming sexual embryos and *N. pseudo-mas*, *N. pseudo-mas* var. *cristatum*, *N. pseudo-mas* var. *polydactyla* Wills, *N. pseudo-mas* var. *polydactyla* Dadds, and *N. filix-mas* var. *polydactylum cristatum* as being "directly" apogamous. MANTON (8) pointed out that these species belong to *Dryopteris filix-mas* (L.) Schott., within which she distinguished three groups corresponding to the three species WOLLASTON (11) separated out of the *filix-mas* complex. MANTON found *Lastrea propinqua* Woll. to have a sporophytic chromosome number of about 80 and sexual reproduction; *L. filix-mas* about 160 chromosomes and sexual reproduction; and apogamous races of *L. pseudo-mas* Woll. about 80, 120, and 160 chromosomes. LANG (7) listed a number of forms of *Aspidium* and *Nephrodium* which are normally sexual but whose prothallia, when fertilization is prevented, produce sporophytic buds.

FARMER and DIGBY (4) reported that in *Lastrea pseudo-mas* var. *polydactyla* Wills and *L. pseudo-mas* var. *polydactyla* Dadds fusions of vegetative nuclei replace gametic union, the result being a diploid cell or group of cells which develop into an embryo. No nuclear fusions occur in gametophytes of *L. pseudomas* var. *cristata apospora*, which is both aposporous and apogamous.

YAMANOUCHI (12) believed he could trace the development of the embryo of *Nephrodium molle* from a single superficial cell located on the ventral side of the prothallium close behind the apical notch. These initials could be detected within 5 weeks after the prothallia reached the two- or three-celled stage. Embryos are likewise formed sexually. HEILBRONN (6) added *Lastrea filix-mas* var. *grandiceps* Woll. to the list of apogamous forms.

STEIL (9) found that in the apogamous species, *Nephrodium hirtipes*, the last premeiotic divisions are incomplete, restitution nuclei with the doubled number of chromosomes resulting. Instead of the usual 16 spore mother cells and 64 spores, 8 and 32, respectively, are formed. STEIL believed this behavior to be indicative of hybrid origin. He found no nuclear migrations in the gametophyte.

STEIL (10) has given a generalized description of the development of embryos of several apogamous species, based on his own and others' observations. The position of an embryo varies from species to species, or even within a species; its location is usually ventral and back of the apical notch, but may be directly in the notch, on a process developed from the notch or on the wings of the prothallium. Generally the prothallial cushion has not developed when the apogamous embryo appears. Tracheids appear in a light green strip of the cushion before the gametophyte has reached full size, and precede the initiation of an embryo in

[1] This work was done in the Barker Cryptogamic Laboratory of the University of Manchester under the direction of Professor W. H. LANG. Appreciation is extended to Professor LANG, DR. IRENE MANTON, and Professor C. E. ALLEN for their interest and constructive criticism. Appreciation is expressed to the National Research Council for its support of this work and that on *Doodia caudata*.

some species. The embryo is usually surrounded by hairs consisting of single rows of cells; in at least two species scales are also present. The apical cell of the leaf differentiates first, next that of the root, and finally that of the stem. No foot is present. The root may outstrip the leaf in development. Secondary prothallia, provided they possess an apical notch, commonly give rise to embryos.

In sporangia of *D. remota* and *D. paleacea* var. *cristata*, DÖPP (2) distinguished three types of behavior of spore mother cells which lead to varying degrees of fertility. Progeny of a mating between sexual *Dryopteris filix-mas* and apogamous *D. paleacea* var. *cristata* resemble the apogamous parent in possessing three types of spore mother cells and in producing apogamous gametophytes. The various sexual forms of the *D. filix-mas* complex he considered to have come from *D. filix-mas* proper; the apogamous varieties, from matings of *D. paleacea* or derived hybrids with forms of *D. filix-mas*. *D. remota*, reported to be apogamous by FISCHER (5), is a hybrid between *D. paleacea* and *D. spinulosa*. MANTON (8) has pointed out that *D. paleacea* Moore is synonymous with *D. pseudo-mas* Woll. Studies of prothallia of *Lastrea pseudo-mas* var. *polydactyla* Dadds and *L. pseudo-mas* var. *polydactyla* Wills led DÖPP to the conclusion that nuclear migrations and fusion therein are obtained only after certain procedures in fixation.

Material and methods

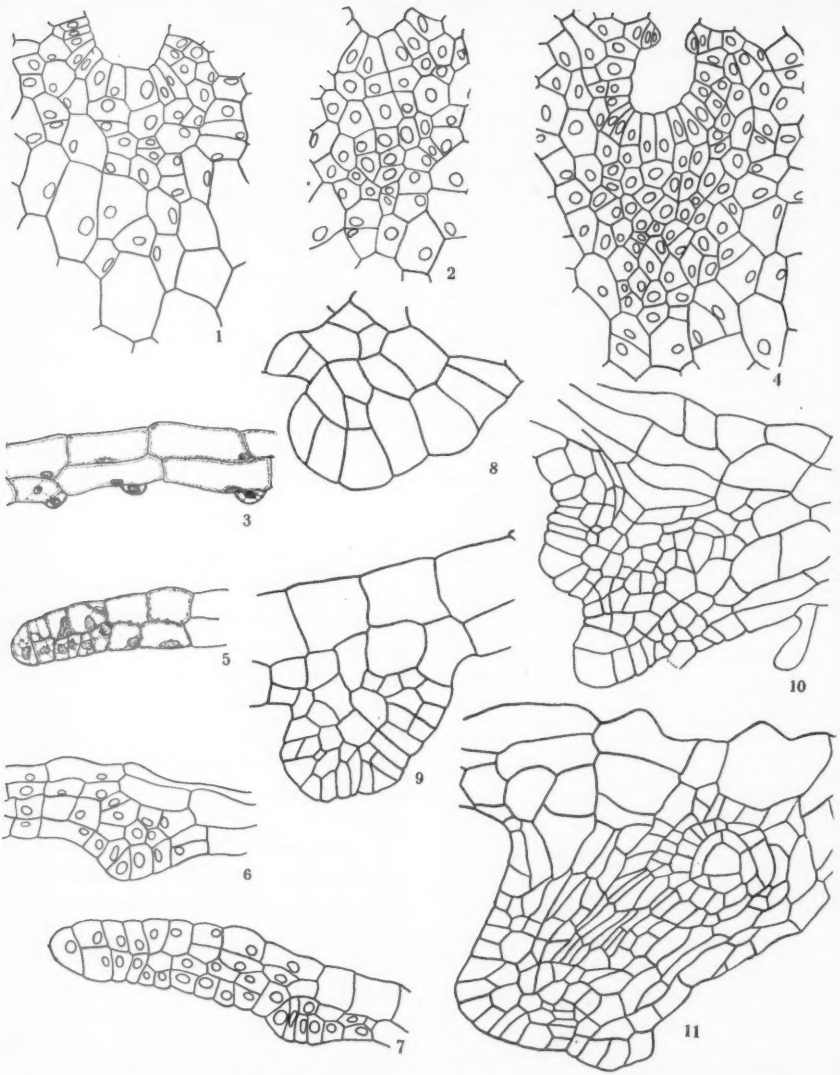
Prothallia of *Dryopteris pseudo-mas* (Woll.), *D. pseudo-mas* var. *polydactyla* Wills, *D. psuedo-mas* var. *polydactyla* Dadds, *D. pseudo-mas* var. *cristata* Cropper, *D. hirtipes* (Bl.) O. Ktze., and *D. mollis* Underwood were studied both in sectioned material and *in toto*. Dilutions of Karpechenko's, Merton 2 BE, and chrom-acetic solutions were used as killing agents. Whole prothallia were mounted in glycerin jelly and balsam. Prothallia bearing embryos and young sporophytes were cleared in Javelle water and stained with decolorized ammoniacal fuchsin. This material was used in the study of the vascular system.

All forms were present at the experimental gardens of the University of Manchester. The *polydactyla* varieties are of the same stock as those used by LANG, FARMER and DIGBY, MANTON, and DÖPP in their researches. Miss MANTON kindly made available the triploid race of *Dryopteris pseudo-mas*.

Observations

Most of the study was devoted to *Dryopteris pseudo-mas*. Both irregular and heart-shaped prothallia are present. The former are without apical notches and do not form embryos but produce antheridia; the latter bear both antheridia and embryos (fig. 14). Several cases of doubtfully incompletely formed archegonia on heart-shaped prothallia were observed.

Young prothallia were studied to ascertain whether nuclear migrations and fusions play a role in the origin of the embryos. In no cases were nuclei found passing through pores in the walls or fusing with other nuclei. Figures 1 and 2 illustrate the cells on the ventral side behind the apical notch of young prothallia at two stages of development prior to initiation of embryos. In the central portion of the prothallium, just behind the developing cushion and anterior to the area in which antheridia and rhizoids may be present in profusion, apparently binucleate cells frequently occur. No empty cells adjoin them. Such a cell examined in section invariably possesses a small projection in which one nucleus



FIGS. 1–11.—Figs. 1, 2, 4, surface views of lowermost layer of cells just behind notch of successively older prothallia; ×200. Fig. 3, vertical longisection of young cushion region of prothallium, showing rhizoid initials; ×150. Fig. 5, vertical longisection through notch region; ×150. Fig. 6, same of embryo arising from cushion four cells thick; ×200. Fig. 7, same of embryo arising from cushion which apparently was two cells thick at time of origin and became four cells thick later; ×200. Fig. 8, lateral surface view of young embryo; ×300. Fig. 9, vertical longisection of embryo arising from cushion two cells thick. Apical cells of stem and leaf present; ×200. Fig. 10, same of young embryo at time leaf outstrips stem in growth; upper two cell layers of cushion little affected; ×200. Fig. 11 same (slightly oblique) of stem of young embryo; apical cell of first root present; ×200.

lies. Darker staining discloses the presence of a wall setting off the projection as a separate cell from the larger upper cell. The small cells so formed are rhizoid initials (fig. 3) and are not found in the area in which an embryo is to arise although they are located immediately behind it. Corresponding hair initials from marginal or superficial cells have similar aspects. There is no evidence of a causal relationship between such cells and the origin of an apogamously formed embryo.

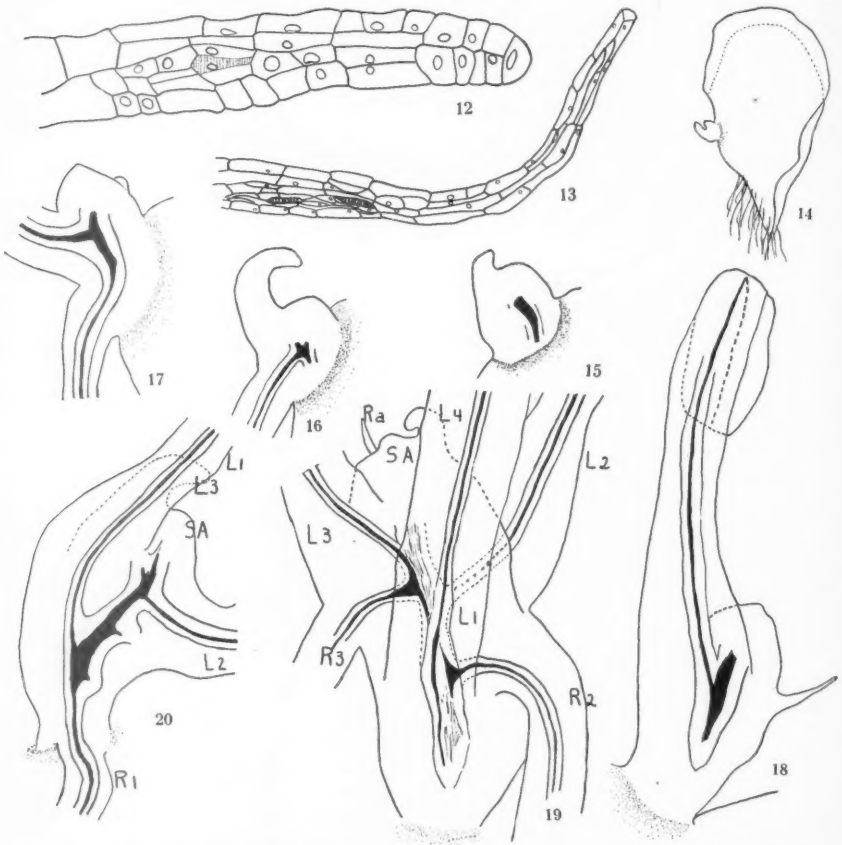
A young heart-shaped prothallium consists of a flat plate one cell in thickness, which is added to by divisions of the cells in the apical notch. When a prothallium reaches a length of approximately 2 mm. and a corresponding width, the cushion becomes two cells thick by division of the cells at a short distance behind the notch. On the ventral side of the cushion of a prothallium is a fairly distinct group of cells (fig. 4) somewhat smaller in size and richer in protoplasm than the adjoining ones. Vertical longitudinal sections of a prothallium at this stage show that the cells in this region on the ventral side are somewhat smaller than those on the dorsal (fig. 5). At least one division occurs in the cells of each layer, the cushion becoming four cells thick. Meanwhile the notch remains active, so that anterior to this thicker region there is a portion of cushion in formation which is as yet two cells thick and in which the cells are somewhat larger. In sections the first indications of apogamous embryo formation observed are cell divisions in layer 3 (the layer next to the lowermost layer in a cushion four cells thick), the resulting cells distend layer 4 (the lowermost layer). Soon the cells of layer 4 divide likewise, in pace with the increase of interior tissue, the planes of the divisions being vertical while most of the interior divisions are in horizontal planes.

Within the layer formed by the divisions of the cells of layer 4 an apical cell is constituted. Figure 6 shows the upper two cell layers of the cushion as yet little affected, the irregular group of cells derived from layer 3, and the appearance of an apical cell in layer 4. The apex diverges slightly forward and downward at about the same angle as lies between the older embryo and the prothallium. If the apical cell does not differentiate and the cushion cells elongate, the interior cells derived from layer 3 elongate likewise and some of them may differentiate as tracheids (fig. 12). Later new prothallial growing points may be constituted along the anterior or inner lateral (next the notch) margin of the wing. An embryo may arise in tissues derived from these new growing points. Embryos have not been observed to originate in the notch tissue directly.

In some cases the embryo arises from a cushion two cells in thickness (fig. 9). Figure 8 illustrates the formation of a small tonguelike bulge following cell divisions on the posterior faces of the apical cell of the newly constituted embryo. Behind such an apex and away from the apical notch of the prothallium a second apical cell arises in one of the segments derived from the first (fig. 9). The original apical cell of the protuberance, the young embryo, is the apical cell of the stem, while the one back along the protuberance is that of the primary leaf. Hairs are now present, growing out from the prothallial cells of layer 4 in the vicinity of the young embryo.

The stem maintains for a time the dominant position shown by the position of the embryo; however, the leaf soon outstrips it in development (fig. 10) and at times slightly displaces the stem to-

ward the notch of the prothallium. By the time the primary leaf has grown well forward and started to bend around the stem apex, considerable stem tissue has resulted from the activity of the apical cell of the stem. The interior cells of this young stem are elongated along the axis lying behind the stem apex. At the base of this young stele, cells derived from layer 3 of the prothallium still possess the power of division. Within the limits of this tissue one cell enlarges considerably more than the surrounding cells and then divides successively in several tangential planes, so that a noticeable sheath is present. This is the apical cell of the primary root (fig. 11). During or immediately before the differentiation of the



FIGS. 12–20.—Fig. 12, vertical longisection of cushion in which internal tissue developed from layer 3 but apical cell failed to appear in layer 4; ×200. Fig. 13, vertical longisection of elongated cushion containing tracheids derived from layer 3; ×100. Fig. 14, young embryo attached to heart-shaped prothallium whose two wings are folded back; ×20. Figs. 15–20, sketches of embryos cleared and stained so that xylem and endodermis are visible. L_1, L_2, L_3, and L_4 are first, second, third, and fourth leaves, respectively. R_1, R_2, and R_3 are first, second, and third roots. SA, stem apex; Ra, ramentum. Figs. 15 and 16, ×40; figs. 17–20, ×50.

root apex, cells of layers 1 and 2 occasionally divide, no great amount of tissue resulting. In the case of one embryo arising from a cushion two cells in thickness, the earliest root departed from the stem at some distance from the insertion of the stem on the prothallium, the root associated with the first leaf being absent (fig. 19). The effect is that of an embryo seated on a stalk; however, the anatomy of the stalk is stemlike.

At about the time of root initiation, the cells of the cushion lying between the apical notch of the prothallium and the point of embryo insertion elongate, the elongation involving generally the meristematic cells at the base of the notch and less frequently those bordering its sides. The result is a general broadening of the notch, so that the wings lie farther apart, and at the same time the wings turn upward. A group of cells from the base of the notch or from the margin of the wing adjoining the notch may push out to form a "middle lobe." There are cases in which a second embryo forms on the portion of the cushion ahead of the first successfully established embryo and, peculiarly, some in which the second embryo lacks the apical cell of a stem derived from the development of layer 4. In this latter case the internal cells (derived from layer 3) of the second embryo, the only evidence of its presence, become involved in cushion changes, and after elongation some become tracheids. Elongation of the cells of the cushion, as well as occasional divisions of the cells of the notch meristem before it loses its embryonic qualities, causes the embryo to lie farther back along the cushion. Once the embryo is well established and elongation of the cushion has taken place, the prothallium concerned has no further development.

An embryo may abort as a whole, or the primary leaf occasionally and the primary root more frequently may abort separately. The earliest abortion occurs at the failure of the first apical cell to appear. In this event the internal cells elongate with the cushion, and some become tracheids, the only evidence of an aborted embryo being the presence of the tracheids in a somewhat thickened cushion (fig. 13). Even though a small protuberance may have been formed through the activity of the first apex, the embryo, particularly as the culture grows older, may fail to continue actively meristematic. Its cells grow larger and become more vacuolate. The differentiation of cells from layer 3 provides tracheids found in elongated cushions more thickened than in the first type of abortion mentioned. Prothallia with such cushions give rise to secondary prothallia, whose origin lies in the meristematic activity of cells just ahead of the elongated cells bordering the wing margin adjoining the prothallial notch, or of marginal cells along the anterior edges of the wing. Secondary prothallia give rise to embryos which may develop or abort. Occasionally an embryo arises early behind a secondary growing point on the inner margin of a prothallial wing. Such an embryo is apparently located on the wing near the notch, since the other wing of the secondary prothallium is as yet small or nonexistent. One arising still earlier and nearer the notch would be classified as arising in the notch, of course; this location has not been observed.

The stele differentiates and matures acropetally in stem, root, and leaf, development of the endodermis keeping pace with that of the protoxylem. In a young embryo bearing one leaf (fig. 15) it is difficult to determine whether the main axis is from root to stem apex or from root to

leaf apex. The stem apex is always present, however; the stele differentiates directly toward it (fig. 16). When the leaf trace has passed out into the leaf base, the vascular system of the stem has extended beyond the insertion of the leaf trace (fig. 17). Older embryos demonstrate that even the first leaf is lateral, since the stem grows directly forward while the first and later leaves diverge from it at about the same angles (fig. 19). When the first root is not present the line of tracheids ceases sharply at the point where the root apex would arise (fig. 18); when it is present the stele of the root is directly continuous with a portion of the stem stele (fig. 17). Occasional tracheids appear in the cushion immediately ahead of the embryo; these are differentiated cells derived from layer 3 of the young prothallium (fig. 20).

Each leaf diverges at an angle of about 100° from the previous leaf (phyllotaxy was not determined), and each is associated with a root which arises endogenously from just below its base. Exceptions to this association are the failure of the first root to appear (fig. 19), or, in the event that this root is present, the frequent abortion of the second root (fig. 20). Primordia of all leaves originate as small moundlike outgrowths among occasional young ramenta on the conical stem tip (fig. 19).

The remaining forms studied have all been treated rather extensively by other workers, so that detailed description is hardly necessary. In general, events seemed to move more or less in the same manner as in *Dryopteris pseudo-mas*. However, certain atypical developments have been thought worthy of listing.

In *Dryopteris pseudo-mas* var. *polydactyla* Wills a relatively massive primary root often develops precociously, even earlier than the primary leaf. In at least one case the primordium of the primary leaf had not appeared, but a well-defined axis between stem and root apex was present.

The tendency toward the development of a strand of tracheids between the embryo and the prothallial notch in *Dryopteris pseudo-mas* var. *polydactyla* Dadds is pronounced. Since at the time of embryo initiation more cells of layer 3 than in triploid *D. pseudo-mas* are involved in the formation of internal tissue, this tissue can occupy a longer portion of the cushion, there being, therefore, greater opportunity for the root to arise at some distance behind the stem or for the tracheidal strand to differentiate forward of the embryo and toward the notch. One compound embryo was observed which consisted of two stems and their associated leaves all related to one primary root.

The prothallium of apogamous and aposporous *Dryopteris pseudo-mas* var. *cristata* Cropper differs now and then from the species in the extension of the internal tissue for a short distance behind the insertion of the embryo. The appearance of the primary root is generally delayed; the first leaf, too, may arise late from a stem which is a cylindrical process terminating in a conical tip. Abortion is frequent. Elongation of the cushion region and segregation of possible growing points along the margin of the prothallium—whether anterior or bordering the notch—lead to formation, by regeneration, of secondary prothallia which are characteristically asymmetrical and produce embryos which chiefly abort. Abortion is generally of the type in which an apical cell does not differentiate in the lowermost layer and in which cells of the interior tissue and of the other layers of the cushion elongate. Middle lobes derived from tissue at the base of the notch occur frequently in the prothallia of this variety.

In *D. hirtipes* the functional association of the tissue derived from layer 3 and of the apex derived from layer 4 is not so close as in the forms previously mentioned. The interior tissue is not so limited and may be formed seemingly independently of any development from layer 4. Among the posterior cells derived from layer 3 the apical cell of the root appears; behind it tracheids differentiate from the stele of the stem. The root may grow out of the cushion tissue, or the root apex may remain in its initial situation. The tendency toward development of tracheids, or at least extension of the endodermis toward the notch region, is common. Two adjacent embryo initials in line with the prothallial notch have been seen. In the course of development they may become related to one primary root. The stem of the posterior embryo is usually abortive and possesses only a few tracheids. Its leaf appears as a lateral outgrowth along the axis between the root apex and the stem of the anterior embryo. In one case the axis of development ran from root apex to prothallial notch and a leaf developed laterally; no stem apex was discernible.

Prothallia of *D. mollis* having only a capillary source of water and of considerable age had peculiar localized thickenings behind the notch region. Sections showed them to be sexually formed embryos which had not erupted from the distended venters of archegonia. No apogamous embryos were observed; the amount of material, however, was limited.

Discussion

De Bary (1) noted that the upper cell layers of the prothallium in *Pteris cretica* are not involved in the apogamously produced embryo which arises from the lower cell layers. These observations are confirmed in this study and are expanded by observations on the divisions of function between the lower two cell layers (layers 3 and 4) of the prothallial cushion as outlined under the treatment of *Dryopteris pseudo-mas*.

The first apical cell is interpreted as that of the stem and the second as that of the primary leaf. This interpretation naturally culminates in the conclusion that the primary leaf of the apogamously produced embryo is lateral in attachment, not first to appear and terminal in attachment as previous workers have indicated. The order of appearance is borne out by comparison of a close series of young embryos (figs. 8, 9, 10, 15, 16). Collateral evidence is the fact that the main axis of differentiation is from the base of the primary root to the stem apex, while the traces leading into the primary leaf and into all later leaves are lateral and not so large as the stele of the stem.

The trace of the primary root originates at the base and toward one side of the vascular system of the stem. Each later root arises next the stele and proximal to the leaf with which it is associated, so that the two traces (of root and leaf) plus the portion of the stele of the stem lying between them form a distinct U. This association of leaf and root is the "phyllorhize" of various investigators, but the present investigation does not support the ultimate conception of the phyllorhize theory that the stem is a collection of traces connecting roots and leaves and that the stem apex arises anew at the base of each new leaf. The results indicate that the stem is a permanent structure, that its apex is present from the beginning, and that the differentiation of tissues within the stem is abreast, ahead many times, of the youngest leaf in whose base a trace is differentiating. Those classes of embryos, al-

though of infrequent occurrence, in which the primary leaf appears late, aborts, or never forms at all so that the stem is conspicuous and those in which the primary leaf appears as the lateral outgrowth of an axis running from the primary root to the notch of the prothallium, the stem having aborted, bear out the contention that the leaf is a lateral appendage.

Several differences between the obligate and induced types of apogamy seem clear. In the obligate type generally a single embryo appears early—at about the time when non-apogamous prothallia are producing archegonia—and arises from a definite area of the prothallial cushion; whereas sporophytic buds of the induced apogamous type, frequently numerous, generally arise late in the development of comparatively larger, more massive, highly modified prothallia, and from no preordained portion of the prothallia. The relative place of tracheid differentiation within the prothallia is characteristic for each type. If the cytological behavior of *Nephrodium hirtipes* or *Aspidium remotum*, described by STEIL (9) and DÖPP (2), respectively, should be found in all the obligate types, then all portions of the life cycle have the same or very similar chromosome number as the result of restitution nuclei formed in the last premeiotic division. The true induced type of apogamy leads to haploid sporophytes produced directly from prothallia with the reduced number of chromosomes, sporogenesis having been normal (3).

The embryo of a sexually produced sporophyte differs from that of one produced normally in that a foot is present. An apogamously produced embryo, however, arises directly from the prothallium and remains connected therewith, no special absorptive structure being necessary. Here may lie an explanation of the involvement of the prothallium in sporophytic tendencies, such as tracheid formation in the cushion. It might be assumed that the changes in question in the cushion are expressions of slightly variable growth-regulatory phenomena. The distinctly sporophytic cells of the embryo and the distinctly prothallial cells, of course, are precisely alike genetically and vary cytologically only in details of cell organization.

Summary

1. The lower two cell layers of the four-layered portion of the prothallial cushion just behind the apical notch are concerned in the initiation of an apogamously produced embryo of *Dryopteris pseudo-mas*. Divisions occur first in the upper of the two ventral layers (layer 3). Before these divisions have ceased, cells of the lower ventral layer begin to divide. The upper two cell layers are not concerned in these changes, although later a few cell divisions may take place. Prothallia whose cushions are two cells in thickness may give rise to embryos, the lower layer behaving as layer 4.

2. The tissue formed from layer 3 gives rise to the root apex and to cells of the prothallial cushion which may become tracheids. Layer 4 gives rise to an apical cell; in a segment derived from it a second apical cell differentiates. The former is the apical cell of the stem; the latter that of the primary leaf.

3. Abortion of the entire embryo, when it occurs, is a consequence of the failure of the first (stem) apex to differentiate. Any particular organ may abort independently of the other organs.

4. Tracheids in the prothallial cushion which bears no embryo are evidences of the abortion of the whole embryo, with

the possible exception of the apical cell of the root, which—except in sectional view—would be indistinguishable. Tracheids lying in the cushion ahead of a developing embryo are evidences that the changes in layer 3 in preparation for embryo initiation involved more of the cushion than the changes in layer 4. Such tracheids arise along an axis between the prothallial notch and the base of the stem.

5. The four forms of the *Dryopteris pseudo-mas* complex and *D. hirtipes* show a highly similar method of apogamous embryogeny.

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ANATOMICAL AND CHEMICAL ASPECTS OF ABSCISSION OF FRUITS OF THE APPLE[1]

MONROE McCOWN

Introduction

Beginning immediately after the petals fall and extending over a period of 5 or 6 weeks, abscission of many of the immature fruits of the apple normally occurs. Abscission of the developing fruits continues, but further shedding of any considerable number of uninjured fruits within a limited period usually does not occur until the approach of fruit maturity. Information on the anatomical development of the tissues involved and the chemical changes in these tissues preceding and during abscission is limited. In this investigation the processes involved in the formation of the abscis layer were studied and are herein described.

HEINICKE (10) concluded that the separation results from the activity of cells near the base of the pedicel. NAMIKAWA (16) reported cell division in the "abscission zone" just prior to the drop of apple fruits. He attributed cell separation to the combined effects of increased turgor and the dissolution of the middle lamella and a part of the secondary cell wall. FEHER (8) also reported that cell division occurs just preceding the separation. MCDANIELS (14) noted the occurrence of cell division in connection with the formation of the "abscission layer" of flowers and young fruits but observed no proliferation of cells preceding the dropping of mature fruits.

METHODS.—The studies were initiated in 1936 and extended over a period of five seasons. An orchard near West Lafayette, Indiana, was the source of the material. The trees, 13 years of age when the studies were begun, produced moderate to heavy crops except in 1938, when a freeze in mid-May destroyed a major portion of the fruit. The collections were made at frequent intervals, from approximately 1 week preceding flower opening until after commercial harvest.

While the varieties Rome Beauty, McIntosh, Grimes Golden, Golden Delicious, Delicious, and Fameuse were included in the microchemical studies and general observations of fresh material, permanent slides were prepared only from samples of McIntosh and Rome Beauty. These samples consisted of small portions of stems embracing parts of both the pedicels and the peduncles of flowers and fruits. The term "peduncle" is applied to that portion of green stem at the tip of the cluster base from which issue the pedicels of flowers or fruits of the apple (13).

The material was left in Rawlin's formalin-acetic acid-alcohol killing solution until needed. Older material was softened in hydrofluoric acid for 5–8 weeks. Dehydration was in ethyl alcohol. The short celloidin schedule was followed in preparing the samples for sectioning. The sections, varying 8–15μ in thickness, were stained in Ehrlich's haematoxylin and safranin and mounted in Canada balsam.

For the microchemical studies and supplementary observations, fresh spurs with pedicels attached were collected and placed in water to prevent drying. All fresh material was studied within a few hours. Freehand sections were cut with a razor blade from the fresh stem portions, except that some fresh flower pedicels were balled in paraffin and sectioned on a microtome. A microscope with disk polarizer and analyzer attachments was utilized in the optical studies.

[1] Journal Paper no. 105, Purdue University Agricultural Experiment Station.

The standard tests were followed in the microchemical studies, as follows:

CELLULOSE.—Hydro-cellulose reaction; cupra-ammonia; polarized light.
PECTIC COMPOUNDS.—Polarized light; ruthenium red; solubility.
PECTIN.—Water-soluble.
PECTIC ACID.—Two per cent KOH.
CALCIUM PECTATE.—Three per cent ammonium oxalate; 2 per cent H_2SO_4 or 2 per cent HCl followed by 2 per cent KOH.
PECTOSE.—Heated for 30–60 minutes in 2 per cent HCl followed by dilute alkali.
LIGNIN.—Phloroglucin-HCl; polarized light (**15, 19, 7, 18**).

Throughout this paper, an effort has been made to conform to the terms suggested by KERR and BAILEY (**12**) and accepted also by ANDERSON (**1**): Middle lamella—the amorphous, isotropic material, largely (if not entirely) pectic compounds first deposited by the cytoplasm. Primary wall—the first anisotropic layer of the wall, composed largely of cellulose and pectic materials. Secondary wall—the additional wall layers formed by the cell.

Investigation

ANATOMY OF PEDICEL

IMMATURE PEDICELS.—At the pink stage of flower development (when the first collections were made), the pedicel was relatively soft. The cells of the various tissues were immature and mostly parenchymatous. In the pericycle some cells were evidently developing as fibers, others as stone cells. A very small amount of secondary xylem and phloem was evident.

Constriction of the base of the pedicel and apex of the peduncle was evident in immature stems (fig. 1). In this constricted zone the cells of all tissues, except those of the pith, were smaller than adjacent ones. This zone was usually 20–30 cells in width in the cortical tissue, less wide in xylem and phloem, and apparently constituted the so-called "abscission zone" of NAMIKAWA (**16**). Since the abscission process is not limited to this zone, however, it seems more appropriate to refer to it as a constriction zone, and it is so designated in this paper (fig. 1).

Enlargement and differentiation of cells in the pedicel proceeded slowly during the period of flowering and shedding of petals. Within a week after petal-fall, fibers and some stone cells in the pericycle, as well as isolated pith cells, exhibited wall thickening and evidence of lignification. Within 3 weeks after the petals fell, many pericyclic and pith cells were well-developed stone cells, and well-formed pericycle fibers were present. Walls of the cells in the constriction zone and in the cortex were much thickened.

No absciss layer is differentiated in pedicels of fruits that remain on the trees after the June drop is completed, and for the purpose of this discussion these surviving pedicels are considered as mature (fig. 2).

MATURE PEDICELS.—During development, cells of the pith, the secondary xylem, and the stone cells and the pericyclic fibers, together with the vessels in the primary xylem, were lignified. The secondary walls of the cortical cells, especially those in the outer and basal portions of this tissue in the pedicel, became much thickened as the result of deposition of cellulose; the thick walls of the small parenchymatous cells characterizing the constriction zone remained chiefly of cellulose.

In the pedicels of fruits of Rome Beauty, Golden Delicious, and Delicious at harvest time, the middle lamella was chiefly pectose, except in the cambial region, cortex, and in the constriction zone —where it was mostly a calcium salt of

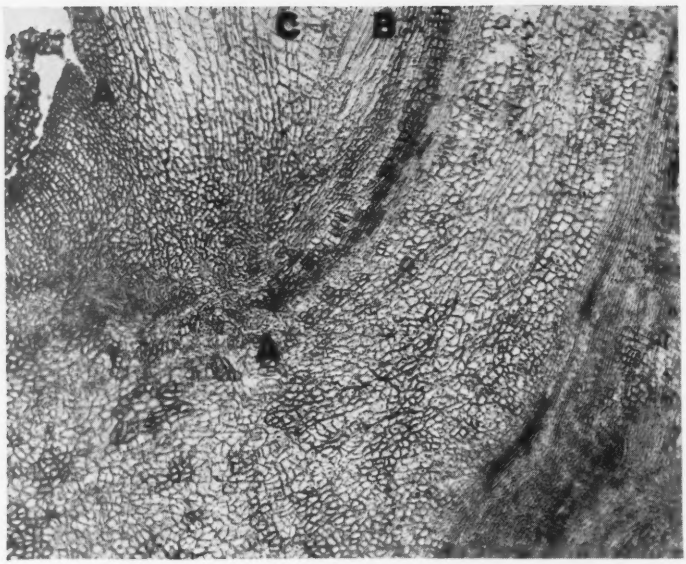

FIG. 1.—Constriction zone of small cells (*A–A*) evident in immature apple pedicel at juncture with peduncle. In pericycle, cells are being modified to fibers (*B*) and stone cells (*C*). McIntosh, 1 week after petal-fall.

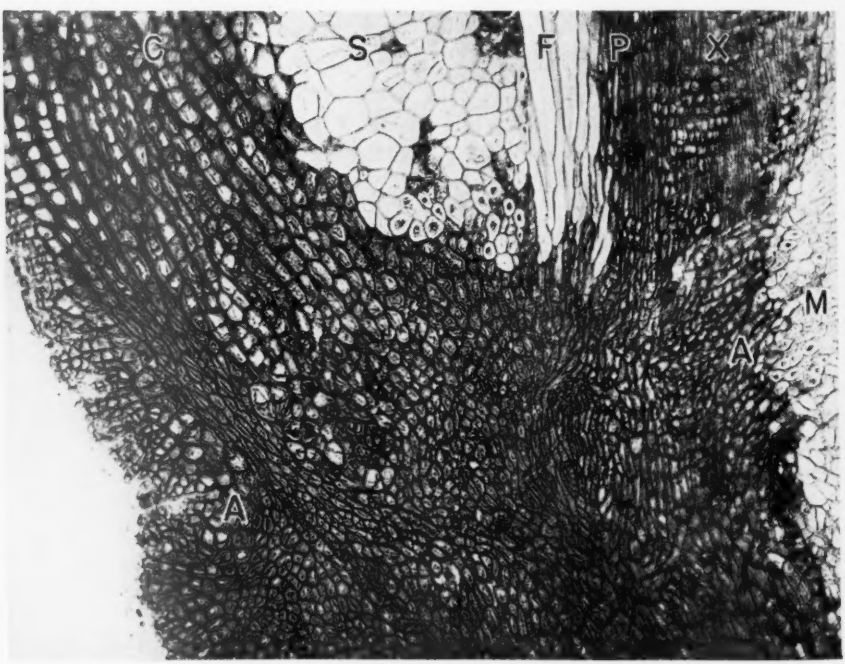

FIG. 2.—Longisection of mature pedicel. At end of June drop, vigorous pedicels appear similar to older one shown here. *C*, cortex; *S*, stone cells; *F*, fibers; *P*, phloem; *X*, xylem; *M*, pith; *A–A*, constriction zone. McIntosh, 15 weeks after petal-fall.

pectic acid. Before the natural process of abscission had reached an advanced stage, separation of the stone cells of the pith could be induced only by heating in weak HCl, indicating the presence of pectose. The walls of the stone cells of the pith were chiefly of cellulose. Extremely thin sections of these cells were tested with phloroglucin-HCl. Very thin lamellae, in close association with the middle lamella and primary wall, and lining the pits and cell lumen, were the only portions of the walls to show positive reaction to this test. In many stone cells of the pith the lignified lamella lining the cell lumen was so thin that it was barely detectable and in some cells was entirely lacking. When treated with iodine and 75 per cent sulphuric acid, the secondary cell wall became swollen and blue and then very slowly disintegrated, indicative of its cellulose nature.

Abscission process

Immature pedicels.—Flowers and immature fruits abscised following differentiation of an absciss layer in the basal portion of the pedicel (figs. 3, 4). This layer resulted from cell division. In the abscising pedicels studied, the layer usually was differentiated within the limits of the constriction zone. In occasional pedicels, however, the layer appeared in the pedicel distal to the constriction zone. This fact has been reported by others (10, 14).

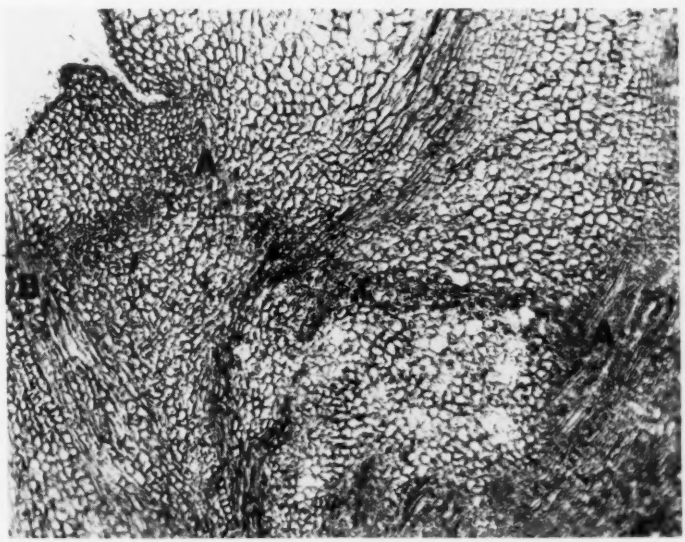

Fig. 3.—Longisection showing differentiation of absciss layer (*A*–*A*) preceding abscission of immature fruit. Contiguous layer (*A*–*B*) has formed across peduncle following abscission of terminal flower. Rome, 1 week after petal-fall.

Cells in all tissues underwent division in the differentiation of the absciss layer, and the resulting layer was six to eight cells in width across the pedicel (figs. 3, 4). Absciss layers were not differentiated in pedicels that survived the June drop.

Following the fall of the terminal flower or immature fruit, the stump of the

peduncle abscised immediately above the most distal lateral flower remaining in the cluster. When all flowers or immature fruits in a cluster dropped, successive portions of the peduncle abscised following the differentiation of a series of two or more absciss layers, the entire peduncle being shed. These layers also were formed by cell division, and in origin and appearance they resembled the absciss layers involved in the shedding of the pedicels (fig. 3).

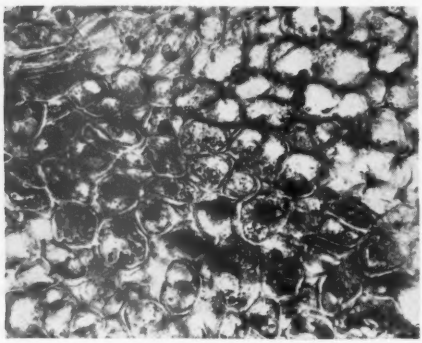

FIG. 4.—Small area of pith from center of section illustrated in fig. 3 showing cell division resulting in formation of absciss layer. Rome, 1 week after petal-fall.

A slight swelling of the middle lamella and primary wall was evident just preceding separation of the cells, which followed disintegration of the pectic compounds of the middle lamella and of the primary wall. There was little evidence of chemical modification of the secondary wall preceding separation. The vessels were ruptured, apparently as the result of mechanical force.

NAMIKAWA (16) also reported the disappearance of the pectic compounds from the walls of cells in the absciss layers of immature apple pedicels during abscission. SAMPSON (17) concluded that the separation of cells in the abscission of coleus leaves results from the dissolution of pectic compounds. APPLEMAN and CONRAD (2, 3), working with peach and tomato fruits, and CARRE (4, 5), CARRE and HORNE (6), and HALLER (9), studying the ripening of apples, concluded that softening of the fruits was due chiefly to the change of insoluble pectic materials to soluble forms. This seems identical with the course of events in the dissolution of the pectic compounds of the middle lamella and primary wall in the abscission of flowers and immature fruits of the apple.

Following the abscising of the pedicel or of the peduncle, cork was formed by a cambium which was initiated a few cells below the surface of the scar.

MATURE PEDICELS.—As stated earlier, pedicels of fruits that survived the June drop were mature, in so far as the mode of abscission is concerned. Division of cells and the consequent differentiation of a well-defined absciss layer were not observed in any mature pedicels. Rather, separation of the pedicel resulted from disintegration of the walls of pre-existing cells. The constriction zone did not determine the path of abscission.

Abscission was initiated independently in the pith and in the cortex of the pedicels studied (fig. 5). In cases where abscission was initiated first in the pith, its course was continued across the xylem, cambium, and phloem, meeting at some point in the bark tissues the line of separation which had arisen in the cortex. If abscission was initiated only in the pith, the separation progressed across the other tissues and was completed by the breaking of cortical and epidermal tissue. The weight of the fruit usually caused the final separation, chiefly by tearing the tissues of the outer portion of the cortex and the epidermis following parting of the inner tissues.

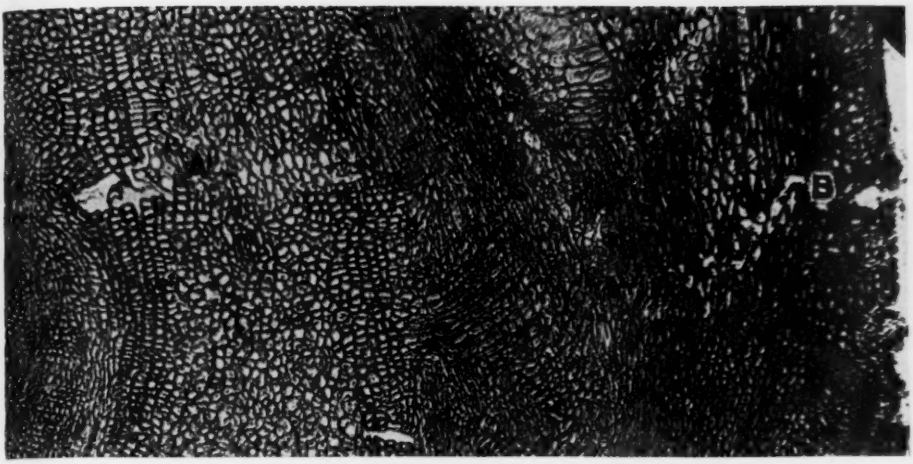

FIG. 5.—Longisection showing abscission of mature fruits initiating independently in pith (*A*) and cortex (*B*). Abscission may be initiated concurrently as illustrated here or begin first in either tissue. McIntosh, 15 weeks after petal-fall.

In some pedicels abscission was initiated first in the cortex, sometimes only in this tissue, and progressed toward the woody cylinder and pith. In which tissue abscission was first initiated, and whether the process was limited to the constriction zone, differed with varieties. The two extremes were in McIntosh and Golden Delicious.

In the mature pedicels of McIntosh, pith abscission usually preceded initiation of the process in the cortex. Separation usually occurred within the limits of the constriction zone. In the pedicels of Golden Delicious, cortical abscission preceded initiation of pith abscission as a rule. In most pedicels of Golden Delicious, abscission occurred in the pedicel distal to the constriction zone, and the line of separation ranged further from this zone as it crossed the pericycle and phloem (fig. 6). Limited observations indicated that the abscission of Grimes Golden pedicels resembled that of McIntosh in this respect. The sequence of pith and cortical abscission in most of the pedicels of Rome Beauty, Fameuse, and Delicious was the same as that in the Golden Delicious pedicels studied.

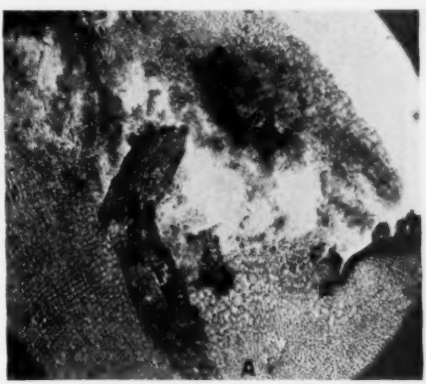

FIG. 6.—Longisection showing advanced stage of abscission in bark tissues of mature pedicel. Separation occurred at base, distal to constriction zone *A–A*, and course ranged upward as it crossed pericycle and phloem. Cells in softer tissues separated at middle lamella, while fibers (*F*) were ruptured. Golden Delicious, 22 weeks after petal-fall.

In the pith of mature pedicels, separation was preceded by more or less swelling and extension of the walls of a few

tiers of cells (fig. 7). Both the stone cells and any parenchyma located in this area became swollen and extended. During the process of swelling and extension of thick walls of the pith cells, the anisotropic quality was lost, indicating a physical change in the cellulose. HODGSON (11) noted this same change in thick-walled cells in abscising citrus leaves.

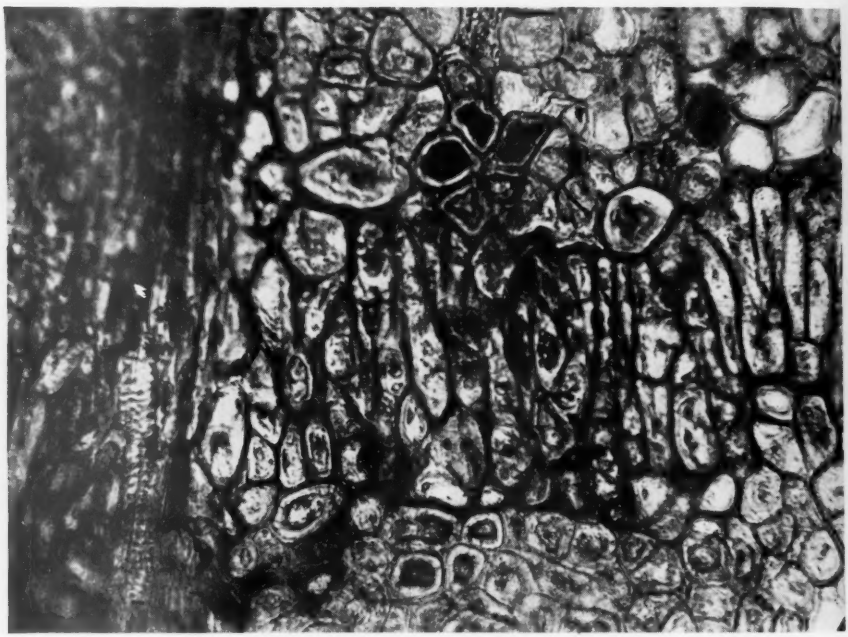

FIG. 7.—Longisection through base of mature pedicel showing cell separation in pith preceded by elongation and re-orientation of a few tiers of cells. Change in pectic compounds in middle lamella indicated by increased prominence of lamella. McIntosh, 15 weeks after petal-fall.

As abscission proceeded, the secondary cellulose wall gradually disintegrated. At the same time the character of the middle lamella was changed. The first indication of this change was an increase in its prominence resulting from swelling, and there was greater intensity of staining with ruthenium red (fig. 7). The pectic compounds of the middle lamella then dissolved, allowing separation of the cells (fig. 8). Just preceding the separation of the swollen cells, a positive test for pectic acid demonstrated a change from insoluble to soluble forms of pectic materials. Following separation, the cells collapsed and the remaining membranes of the disintegrating pith cells were made up of the lignified lamellae and a thin remnant of the secondary wall (fig. 8).

In tissues other than the pith, less disintegration of the cellulose of the secondary wall accompanied dissolution of the pectic compounds. Vessels and fibers in the path of abscission were ruptured by mechanical stress.

Following separation of the pedicel, the cells at the surface of the wound

dried and collapsed. Within 2 weeks the wound was well protected by cork.

Summary

1. A constriction zone, evident in the flower pedicels, persisted during the life of the apple fruits studied but did not function directly as an abscis layer or predetermine the course of abscission in pedicels of flowers or fruits.

2. The abscission of flowers and of immature fruits was preceded by differentiation of an abscis layer. Cell separation followed dissolution of the middle lamella.

3. Abscission of mature pedicels was initiated independently in the pith and cortex and not preceded by cell division.

4. The thick walls of the stone cells of the pith of mature pedicels were made up largely of cellulose lamellae. Separation of these cells was preceded by swelling and extension of the walls. During this process these cell walls lost their anisotropic quality, indicating a change in the nature of the cellulose.

5. In the course of cell separation in the pith, dissolution of the pectic compounds of the middle lamella and disintegration of the primary and much of the secondary cell walls occurred, leaving the lignified lamella and remnants of the secondary wall. In the separation of cells in tissues other than the pith, less disintegration of the secondary wall accompanied dissolution of the middle lamella and primary wall. Vessels and fibers in the path of separation were ruptured.

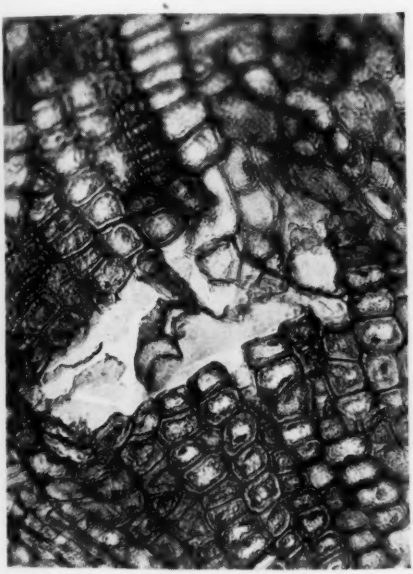

FIG. 8.—Enlargement of portion of pith area of section illustrated in fig. 5 showing dissolution of cell walls and advanced stage of pith abscission in mature pedicel. Remaining membranes of disintegrating cells, now supported by celloidin film, represent chiefly thin lignified lamellae and remnants of secondary walls. McIntosh, 15 weeks after petal-fall.

The writer is indebted to DR. J. H. GOURLEY and DR. H. C. SAMPSON, Ohio State University, for valuable advice and suggestions during the course of the study; also to the Departments of Biology, Forestry, and Horticulture, Purdue University, where the major portion of the work was done, for laboratory facilities.

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CYTOLOGICAL OBSERVATIONS IN CITRUS: III. MEGASPOROGENESIS, FERTILIZATION, AND POLYEMBRYONY

OSWALDO BACCHI

Introduction

An extensive program of investigation of the genus *Citrus* has recently been organized by several divisions of the Instituto Agronômico, its Limeira Citrus Experiment Station, and the Agricultural College at Piracicaba—all located in the State of São Paulo. The present paper discusses the megasporogenesis and embryology of *Citrus;* and a new type of polyembryony, not yet described for this genus, is presented.

STRASBURGER (**10**) was the first to make detailed cytological investigations in this genus. He not only described microsporogenesis and megasporogenesis, fertilization and endosperm formation, but he also explained the formation of adventitious embryos ("sporophytic polyembryony"), first discovered in 1719 by LEEUWENHOEK. The conclusions of OSAWA (**6**), who investigated *Poncirus trifoliata* and the varieties Unshiu (*C. nobilis* Lour.) and Washington Navel (*C. sinensis* Osbeck), agreed with STRASBURGER'S findings as to the origin of the adventitious embryos, which were shown to develop from cells of the nucellus surrounding the megagametophyte. FROST (**4, 5**) proposed the hypothesis of cleavage polyembryony to explain the production of two hybrid seedlings from a single seed, as among 1200 hybrids he found ten such pairs which gave rise to apparently identical twins.

Similar cases were found by TRAUB and ROBINSON (**14**); from 782 seeds from a cross between lemon and trifoliata orange, sixteen produced two hybrid seedlings, one three, and another four hybrids. They state that twin hybrids were also found by SWINGLE in 1909.

Various aspects of polyembryony in *Citrus* and related genera (*Poncirus* and *Fortunella*) have also been investigated (**9, 12, 15–17, 13**), and a revision of the subject has been presented by J. M. WEBBER (**18**). So far as known, only two types of polyembryony have been reported for this group of genera, nucellar and cleavage (**4, 5**), the latter caused by "fission of the generative (sexually produced) embryo."

MATERIAL AND METHODS.—Material was collected at the Citrus Experiment Station at Limeira of the Instituto Agronômico, from representative trees of two species, Grapefruit Foster (*Citrus paradisi* Macf.) and Sour Orange (*C. aurantium* L.).

For the study of megasporogenesis, ovaries were collected from buds at different stages of development and also from recently opened flowers. For other observations the ovaries were collected at intervals from flowers emasculated before anthesis and artificially pollinated 4 days later.

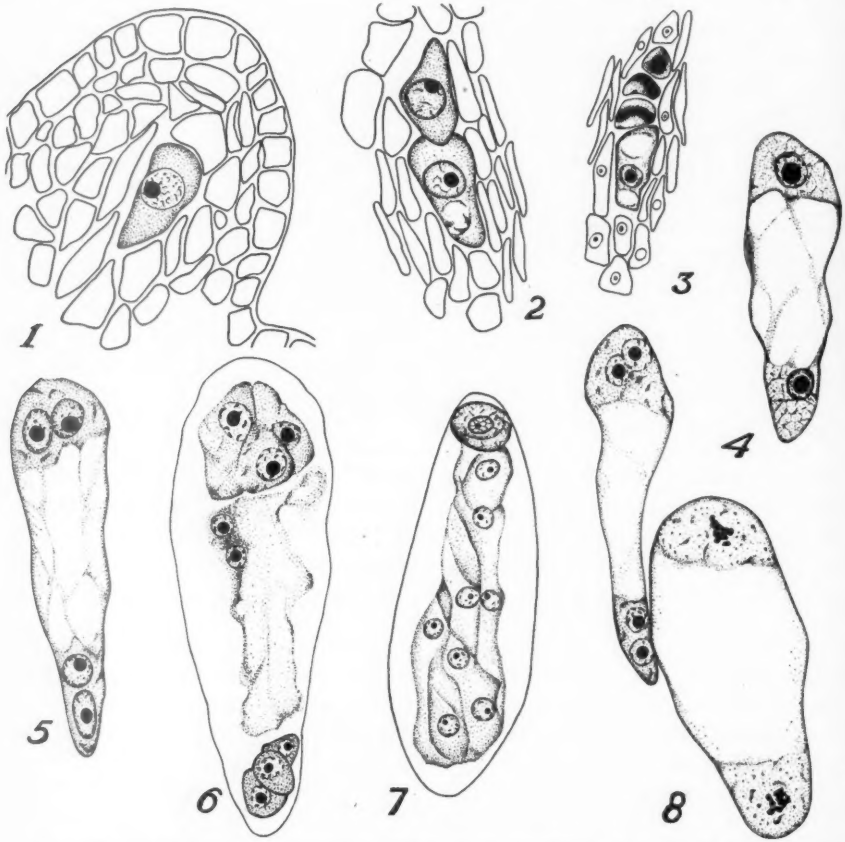
Fixation of the ovaries, which were dissected to insure a better infiltration of the fixing fluid, was made in Craf (**7**). After 24 hours the material was washed in 70 per cent alcohol, dehydrated by the butyl-alcohol method, and imbedded in paraffin. Sections were cut at 10–25 μ and stained in Heidenhain's haematoxylin.

Observations

MEGASPOROGENESIS.—The megaspore mother cell gives rise to a linear tetrad, and the three micropylar megaspores degenerate, the chalazal one forming the functional megaspore, from which a normal megagametophyte (figs. 1–6) develops. There are no differences between the two species studied. Two well-developed synergids occur near the micropyle. The egg lies between the two syner-

gids, a little farther away from the micropyle; in size it is similar to the synergids, except that its cytoplasm contains some inclusions absent in the former. The two polar nuclei are located in the middle of the megagametophyte; usually they remain separated until fertilization, sometimes fusing a little earlier. The three small antipodals degenerate very early.

As a general rule, the first meiotic division (fig. 1) occurs when the ovule is not yet fully developed. The megagametophyte is mature a few days before or by the day the flower opens (fig. 6). In some instances, however, opened flowers have the megagametophyte in the one-, two-, and four-nucleate stages. Usually megasporocytes and megagametophytes

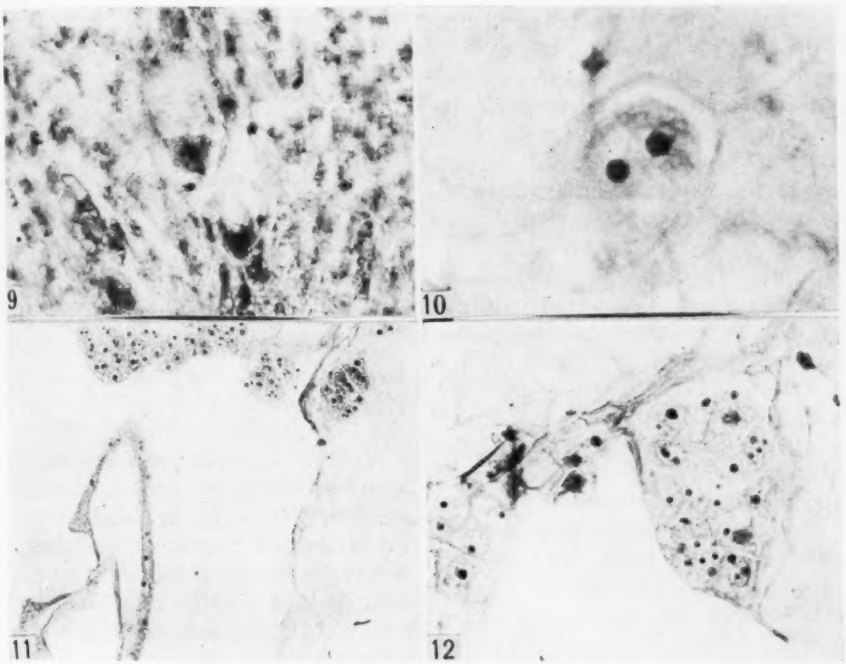


FIGS. 1–8.—Fig. 1, Grapefruit Foster: megasporocyte in prophase from ovary of small flower bud. Fig. 2, Grapefruit Foster: two megaspores in ovary of small flower bud. Fig. 3, Sour Orange: four megaspores, three micropylar ones degenerating; ovary of large flower bud. Fig. 4, Grapefruit Foster: megagametophyte with two nuclei; ovary of large flower bud. Fig. 5, Grapefruit Foster: megagametophyte with four nuclei; ovary of large flower bud. Fig. 6, Grapefruit Foster: complete megagametophyte; ovary of flower bud the day it opened. Fig. 7, Grapefruit Foster: egg cell in resting stage and endosperm with 8 nuclei; ovary 15 days after pollination. Fig. 8, camera-lucida drawing of fig. 9, including observations of adjoining sections. Figs. 1–6, 8, ×1000; fig. 7, ×500.

in different stages of development are found in the same ovary.

FERTILIZATION.—STRASBURGER (10) and OSAWA (6) state that fertilization usually occurs 4 weeks after pollination. In the Grapefruit Foster it was established that the period between pollination and fertilization averaged 4 days. This agrees with COIT's (3) statement that "the time required for complete fertilization after pollination varies with the varieties from thirty hours in the Satsuma Orange to four weeks in the trifoliate orange."

The pollen tube enters the megagametophyte through the micropyle, usually disorganizing one of the synergids. Sometimes both of them disappear at this time. In rare instances they remain untouched. The antipodals soon degenerate; the zygote then initiates a rather long resting period of about 50 days. This period has been found by OSAWA (6) to be 3–4 weeks in *Poncirus trifoliata*.



FIGS. 9–12.—Grapefruit Foster: Fig. 9, development of two megagametophytes in same ovule; ovary day the flower opened. Fig. 10, nucellar cell after first division giving rise to adventitious embryo; ovary 67 days after pollination. Fig. 11, nucellar embryos and endosperm nuclei; ovary 67 days after pollination. Fig. 12, detail of developing nucellar embryo; ovary 67 days after pollination. Figs. 9, 12, ×500; fig. 10, 1000; fig. 11, ×250.

Soon after its formation, through fusion of the polar nuclei with one of the generative nuclei from the pollen tube, the primary endosperm nucleus divides, and successive divisions result in a free nuclear endosperm (figs. 7, 11). Examination of ovaries collected up to 67 days after pollination showed that in some cases the endosperm nuclei numbered more than 1500. Later stages of endo-

sperm development were not examined, so that the formation of cell walls and subsequent cellular divisions reported by OSAWA (6) and by STRASBURGER (10) were not observed.

POLYEMBRYONY.—When the first divisions of the zygote occur to form the embryo resulting from fertilization, several nucellar cells (fig. 10) in the vicinity of the embryo sac, and usually close to the micropyle, through a series of divisions form a number of adventitious embryos (figs. 10–12), which penetrate the cavity of the embryo sac. Such a process, described by others in detail (10, 6), has been observed in young seeds of both varieties studied.

In an examination of many ovules, four instances were detected in which two normal gametophytes developed in the same ovule; the fertilization of their egg cells would therefore have given rise to the development of two generative embryos in the same seed. Figures 8 and 9 show stages of the development of such supernumerary gametophytes.

The occurrence of two gametophytes in the same ovule has already been described for several other genera: *Hiptage* (11), *Medicago* (8), *Poa* (1), and *Saxifraga* (2). With the exception of FROST'S (4, 5) identical twin hybrid *Citrus* seedlings, it is not known whether the other groups of hybrids derived from single seeds, as obtained by TRAUB and ROBINSON (14) and by SWINGLE, in 1909, were genetically identical.

To establish a hypothesis regarding the origin of twin hybrids, it is necessary first to determine whether they are genetically identical. If not, the occurrence of two gametophytes in the same ovule is the best explanation. When more than two hybrid seedlings develop from the same seed, their origin can be accounted for either through cleavage polyembryony alone or through this phenomenon combined with the existence of two gametophytes in the same ovule, as the occurrence of more than two is highly improbable.

Summary

1. A general review is presented of megasporogenesis, fertilization, endosperm formation and polyembryony in *Citrus*, most of the findings of STRASBURGER (10) and OSAWA (6) having been confirmed through investigation of *C. paradisi* Macf. and *C. aurantium* L.

2. A new form of polyembryony is presented, caused by the existence in some instances of two gametophytes in the same ovule. The origin of two non-identical hybrids from the same seed is thus explained.

3. Three forms of polyembryony are therefore known in *Citrus:* (*a*) nucellar embryony, giving rise to a variable number of identical, "maternal" seedlings derived from the nucellus; (*b*) cleavage polyembryony (4, 5) originating through fission of the generative embryo; and (*c*) polyembryony caused by the occurrence of more than one normal gametophyte in the same ovule. The endosperm in *Citrus* is free.

The writer is indebted to Messrs. C. A. KRUG and A. J. T. MENDES for help with the English in writing this paper.

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STUDIES ON AMBROSIA: III. PISTILLATE AMBROSIA ELATIOR × A. TRIFIDA AND ITS BEARING ON MATROCLINIC SEX INHERITANCE[1]

KENNETH L. JONES

Introduction

In nature *Ambrosia elatior* L. is predominantly monoecious. The staminate flowers are borne in small heads arranged in racemes, and the pistillate flowers occur in the axils of leaves or bracts. Rarely the racemes are pistillate or androgynous. The writer has shown (4) that the inheritance of floral types is controlled by the female parent. For example, the common monoecious type breeds true generation after generation, irrespective of the genotype of the pollen parent, and pistillate plants always yield a mixed progeny of monoecious, intergrade, and pistillate. On the other hand, *A. trifida* L. is strictly monoecious in nature, identical with the common type of *A. elatior*.

WYLIE (7) reported a natural hybrid between the species. On the basis of this compatibility, supported by success in crossing *A. bidentata* and *A. trifida* (3), interspecific crosses were attempted to study the inheritance of the pistillate character. It was planned to cross reciprocally *A. trifida* and nearly pistillate *A. elatior*, but attempts to use *A. elatior* as a male parent have failed for 3 consecutive years. The cross reported here between pistillate *A. elatior* and *A. trifida* gives additional evidence of the peculiar matroclinic sex inheritance in *A. elatior* and incidentally offers interesting material for meiotic studies because of specific differences in the size and number of chromosomes.

Results

Seventy pistillate plants of *A. elatior* were grown amidst monoecious *A. trifida* in the greenhouse at the University of Michigan. Planting was sufficiently early to avoid the "ragweed season" in nature. Only seven seeds developed, and these were after-ripened at 5° C., as DAVIS (1) found necessary for this genus. The seeds were sown in early May, together with some of the parent species.

The foliage of the hybrid plants was clearly intermediate in type. Figures 1–3 show typical leaves of the parent species and of a hybrid, from plants grown under similar conditions in the greenhouse. The three-lobed habit of *A. trifida* is evident in the hybrid, but the lobes are considerably reduced and the terminal one pinnatifid. Some leaves have the serrate margin of *A. trifida*. The surfaces are puberulent, the lower surface slightly lighter in color.

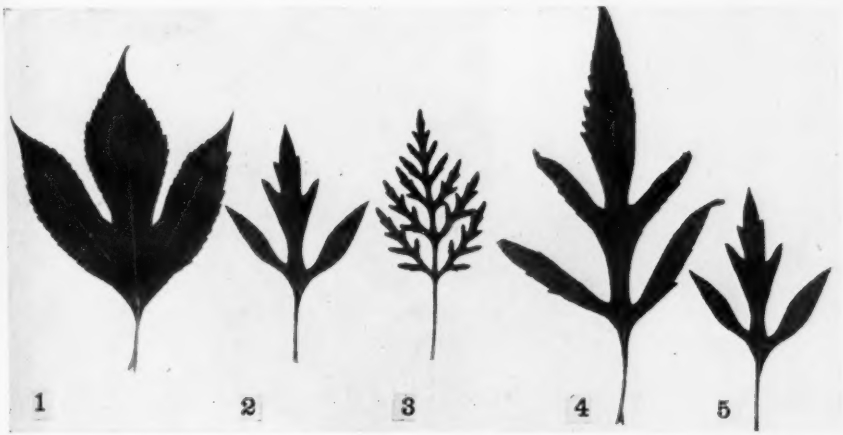
WYLIE (7) illustrated leaves of a natural hybrid collected near Iowa City. They were 2 dm. long and 1.5 dm. wide and were 3-divided with pinnatifid segments. The writer also found a specimen of a natural hybrid on the bank of the Huron River near Ann Arbor in 1936. One of its leaves (fig. 4) is shown with a leaf of an artificial hybrid (fig. 5) for comparison. Its leaves were about 1.5 dm. long and 0.8 dm. wide, whereas the artificial hybrids were only 1 × 0.6 dm.

Chlorotic areas appeared in leaves of all plants from the outset. The deficiency was slight in plants 1, 5, and 7; more marked in 2, 4, and 6; and extreme in plant 3. Plants 1 and 6 had wrinkled leaves which suggested a mosaic disease. These abnormalities were perhaps the effects of recessives for which there were no allelomorphs.

The hybrids grew slowly. On July 11,

[1] Paper from the Department of Botany, University of Michigan, no. 731.

A. trifida was 6.5–7 dm. tall and *A. elatior* mostly about 3.0 dm., whereas the hybrids were only 1.4–3.0 dm. Pollination began on July 1 for *A. elatior*, July 9 for *A. trifida*, and August 17 for the hybrids. On July 21, hybrid plants 1, 2, 3, 4, and 7 were transplanted to the field with those of the parent species. The maximum heights at the end of the season were: *A. trifida* 9 dm. in the field and 12 dm. in the greenhouse; *A. elatior* 4.4 and 7 dm.; and convenience, the types will be designated pistillate, monoecious, and intergrade. The great diversity of the intergrades is illustrated in figures 10–14, in which their racemes are arranged in a progressively staminate series, as follows: figure 10, pistillate except for a few small staminate heads at apex; figure 11, pistillate except in upper left portion; figure 12, pistillate and staminate in about equal proportions—the pistillate in lower half; figure



FIGS. 1–5.—Fig. 1, *A. trifida*. Fig. 2, *A. elatior*×*A. trifida*. Fig. 3, *A. elatior*. Fig. 4, natural hybrid. Fig. 5, *A. elatior*×*A. trifida*.

the hybrids 5.5 dm. under both environments. The poor growth of the parent species outdoors was due to their maturity when transplanted. All hybrid plants were self sterile and failed to produce seed when pollinated by the parent species. The natural crosses observed by WYLIE and the writer also formed no seed.

The plants were of three sex types, exactly comparable with the offspring of a pistillate plant within the species *A. elatior*, namely: (*a*) pistillate (fig. 7); (*b*) monoecious with staminate racemes (fig. 8); and (*c*) monoecious intergrades with androgynous racemes (figs. 10–14). For 13, staminate except for a cluster of pistillate heads in submedian portion; and figure 14, staminate but for two small pistillate heads at apex.

HYBRID PLANTS

PLANT 1, PISTILLATE.—Heads subtended by leafy bracts 2 cm. long (fig. 7); in some racemes the terminal 0.5 cm. lacked bracts. Racemes 5–7 cm. long. Height 4.4 dm.

PLANT 2, INTERGRADE.—Racemes predominantly staminate. Most racemes staminate except for a few sterile pistillate heads at apex (fig. 14). Some entirely staminate and others only half stami-

nate, with pistillate heads subtended by long bracts (fig. 12). Racemes 6 cm. long. Height 5.5 dm.

PLANT 3, PISTILLATE.—Poorly developed plant with general habit like plant 1. Leaves extremely chlorotic. Height 4.4 dm.

PLANT 4, INTERGRADE.—Racemes predominantly staminate. A few inflorescences had occasional pistillate heads; these were never terminal and seldom bore long bracts. This plant most closely resembled *A. elatior*. Racemes 8 cm. Height 3.5 dm.

PLANT 5, INTERGRADE.—Racemes predominantly pistillate. The only staminate heads occurred at the apex of one short raceme. Pistillate heads all subtended by short bracts, less than 1 cm. in length. Racemes 18 cm. Height 4.8 dm.

PLANT 6, INTERGRADE.—Racemes predominantly pistillate. The only staminate heads occurred in lower portion of central raceme. Pistillate heads seldom subtended by bracts. Height 5.5 dm.

PLANT 7, MONOECIOUS.—Racemes entirely staminate (fig. 8), pistillate flowers in leaf axils. Racemes 11 cm. long. Height 4.6 dm.

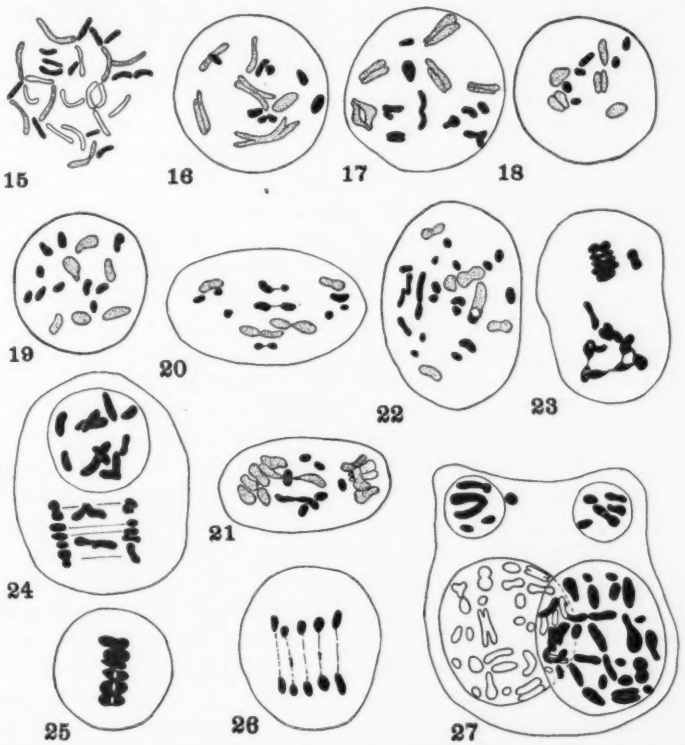
CYTOLOGY

Root tips were fixed in Navashin's and Bouin's reagents and the sections treated with iron-alum haematoxylin. Satisfactory cytological material was obtained, except for plant 6. The writer previously reported (**4, 3**) that *A. elatior* has a haploid chromosome number of 18 and *A. trifida* 12, with the chromosomes larger in the latter species. The somatic figures confirmed the hybrid origin. For example, figure 15 is a typical late prophase with 30 chromosomes, of which 13, filled in solid, are identifiable as *A. elatior*, and 7, stippled, as *A. trifida*. Somatic counts were as follows: plant 1, 26–31

FIGS. 6–14.—Figs. 6–9, racemes of *A. elatior*×*A. trifida* and parents. Fig. 6, pistillate raceme of *A. elatior*. Fig. 7, same of hybrid (plant 1). Fig. 8, staminate raceme of hybrid (plant 7). Fig. 9, same of *A. trifida*. Figs. 10–14, androgynous racemes of hybrids in progressively staminate series. Fig. 10, pistillate except for a few staminate heads at apex. Fig. 11, pistillate except in upper left portion. Fig. 12, pistillate in lower half and staminate in upper. Fig. 13, staminate except for submedian portion. Fig. 14, staminate except for two heads at apex.

chromosomes; plant 2, 29; plant 3, 30–33; plant 4, 26–31; plant 5, 28–30; and plant 7, 31. Exact counts were often impossible because of the overlapping of curved segments.

Racemes of plants 2 and 7 were fixed in Carnoy's fluid and sections stained with crystal violet-iodine. No attempt was made to interpret early prophases. Diakinesis revealed regularly an autosynapsis within each species. For *A. trifida* this was very marked; often only bivalents were found. The writer is uncertain that autosynapsis is ever complete



FIGS. 15–27.—Fig. 15, prophase of somatic mitosis in root tip. Thirty chromosomes: 13 filled in solid (*A. elatior*); 7 stippled (*A. trifida*); and 10 clear (unidentifiable). Figs. 16–22, meiotic division I, showing portion of chromosomes only. *A. trifida* stippled and *A. elatior* solid. Fig. 16, diakinesis; 4 bivalents of *A. trifida*, 1 with heteromorphic chromosomes. Possible instance of allosynapsis. Fig. 17, diakinesis; 5 bivalents of *A. trifida* and also of *A. elatior;* 4 univalents of *A. elatior*. Fig. 18, typical polar view of metaphase. Fig. 19, metaphase in polar view showing 6 chromosomes of *A. trifida* and 10 of *A. elatior*. Fig. 20, anaphase: 5 autosynaptic mates undergoing separation, 2 of *A. trifida* and 3 of *A. elatior*. Near each pole are 4 univalents, 1 of *A. trifida* and 3 of *A. elatior* which presumably have disjoined. Fig. 21, anaphase; 5 univalents of *A. trifida* at each pole, and 1 bivalent undergoing separation. Eight chromosomes of *A. elatior;* 1 bivalent being separated and 6 univalents disjoined earlier. Fig. 22, anaphase; disjunction of chromosome of *A. elatior* from end of its *A. trifida* partner. High chromosome number (21) of *A. elatior* indicates division of unpaired univalents. Figs. 23–27, meiotic division II; all chromosomes filled in solid since size differences were slight except in telophase. Fig. 23, metaphase above, prophase below. Fig. 24, lagging of chromosomes. Fig. 25, metaphase, illustrating regularity of division II. Fig. 26, anaphase; almost diagrammatically equational. Fig. 27, telophase; total of 70 chromosomes indicates division of some univalents in I and II; irregularity of one division and equational nature of other corroborated.

in *A. trifida*, since the sections were too thin to show entire nuclei and the highest number of bivalents observed was 5 (fig. 17). Also, allosynapsis occurred infrequently. Pairing within *A. elatior* was less common, but there were always some bivalents, up to as many as 5 (fig. 17). Synaptic mates were usually similar but heteromorphism was not rare (figs. 16, 17).

The first meiotic division was very irregular and extremely difficult to interpret. The division involved primarily a separation of autosynaptic mates and a random segregation of unpaired univalents. A few univalents underwent equational division in certain microsporocytes. Characteristic polar views of metaphase revealed only a portion of the total number of chromosomes (fig. 18). Figure 19 is unusual in this respect, since 16 chromosomes appear, including 6 of *A. trifida* and 10 of *A. elatior*. The most critical observation made for division I is illustrated in figure 20. At the equator are 5 autosynaptic bivalents undergoing separation, 2 of *A. trifida* and 3 of *A. elatior;* near each pole are 4 univalents, 1 of *A. trifida* and 3 of *A. elatior*, which presumably have disjoined. Figure 21 is less convincing. At each pole are 5 univalents of *A. trifida*, 1 bivalent of *A. trifida* is under the stress of disjunction, and the rest of the field shows 8 chromosomes of *A. elatior;* 1 bivalent is being separated and 6 univalents have apparently disjoined earlier. Figure 22 illustrates one of the few instances of the separation of allosynaptic mates. A small chromosome of *A. elatior* is disjoining from the end of its *A. trifida* partner. Lagging of chromosomes occurred rarely, except in certain anthers where it was extreme in every meiocyte. Amitosis was similarly restricted, so that localized physiological phenomena must bring on these abnormalities.

Meiotic division II seemed to follow directly without delicate reticula developing in the interphase. Figure 23 shows one of the few prophases with anastomoses. The second division is as regular as the first is irregular. The chromosomes line up with diagrammatic neatness at the equatorial plate, and all undergo equational division at almost the same moment (figs. 25, 26). The chromosomes are much shortened and thickened, so that they cannot be identified as to species. The only aberrant phenomenon was an occasional lagging of chromosomes (fig. 24).

The telophase nuclei were grouped usually in quartets, with sister nuclei having approximately the same chromosome numbers. In about 50 per cent of the quartets, one pair of nuclei had very different chromosome numbers from the other. The total number of chromosomes within a quartet seldom exceeded 60, which confirmed the observation that unpaired univalents rarely divide in division I. Figure 27 shows an exceptional instance in which 70 chromosomes occurred; it also illustrates the irregularity of one division and the equational character of the other. Four pollen grains of unlike size ordinarily developed from a sporocyte.

Discussion

The writer has reported (4) that the sex inheritance in *A. elatior* is controlled by the female parent. For example, a pistillate plant, regardless of the genotype of the male, produces a high proportion of pistillate offspring and a motley assemblage of monoecious segregates. It is significant that these results were obtained when pistillate plants were crossed with *A. trifida*, which in nature is strictly of one monoecious type.

MANN (6) has reported that a short photoperiod may cause pistillate flowers

of *A. trifida* to develop in positions usually occupied by staminate, and he states that "In *A. trifida*, on the other hand, length of photoperiod and number of photoinductive cycles have a pronounced effect on sex expression. Lacking experimental information, the uniformity in field plants would suggest that genetic factors are of less importance in *A. trifida* than in *A. elatior*."

Broad comparisons of the relative importance of environment and heredity are always fraught with difficulty. The uniformity of *A. trifida* in the field may be as genetical in its causes as is the variability of *A. elatior*. From the environmental angle, the experimental work has not been comparable, but there is evidence that *A. elatior* of certain strains is alterable in sex. GARNER and ALLARD (**2**) grew plants under a photoperiod of 7 hours from June 3 to July 1, when pollination began in normal staminate racemes. The plants were restored to natural light conditions and blossomed a second time in August, producing "pistillate flowers almost exclusively." The writer (**5**) grew known genetical strains under long photoperiods and found that some were altered and others were not. Strain P, for example, showed an increase of 24 per cent pistillate plants under a photoperiod of 20 hours. This was perhaps equal to the most extreme case cited by MANN for *A. trifida*, "in which some or all staminate involucres were replaced by pistillate involucres" in thirteen terminal racemes and eight lateral out of 100 plants.

Deviations were always toward the pistillate condition, and certain unusual modifications occurred in both species, such as the development of leaves in the staminate racemes (cf. **6**, fig. 4*B* and **5**, fig. 16). The number of hybrid plants was too small for genetical analysis; however, the wide range of types suggests that sex is of a quantitative nature, influenced by many genes. There is no question of the transmission of the pistillate character by the female gametes of *A. elatior*.

The following chromosome numbers have been determined by the writer for *Ambrosia: A. trifida* n = 12, *A. bidentata* n = 17, *A. elatior* n = 18, *A. trifida* × *A. bidentata* 2n = 29 (**3**), and *A. trifida* × *A. elatior* 2n = 30. On the basis of these numbers and of the rather complete autosynapsis among chromosomes in the haploid complement of *A. trifida*, it is probable that the basic number for the genus is 6.

Summary

1. Pistillate plants of *A. elatior* always yield a mixed progeny of monoecious plants, intergrades, and pistillate individuals, irrespective of the genotype of the pollen parent.

2. Pistillate plants were crossed with *A. trifida*, which is strictly monoecious in nature. Seven hybrids were secured: two pistillate, four intergrades, and one normal monoecious.

3. The hybrids had approximately 30 somatic chromosomes (*A. trifida* n = 12, *A. elatior* n = 18). The first meiotic division was extremely irregular and involved primarily a separation of autosynaptic mates and a random segregation of univalents. The second division was regular and equational.

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EFFECTS OF TEMPERATURE ON RUBBER ACCUMULATION BY THE GUAYULE PLANT

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Introduction

Preliminary experiments in which plants of guayule (*Parthenium argentatum*) were cultivated in the greenhouse under various conditions for varying periods showed that such plants failed to form any considerable amount of rubber; generally it was less than 0.5% of the dry weight of the defoliated plant. The following environmental factors failed materially to increase the percentage of rubber in such greenhouse plants: (1) drought of varying degrees, (2) deficiency of nitrogen to varying extents, (3) day length. The data to be presented here, however, indicate that the temperature to which the plants are subjected is an important factor in influencing rubber formation by young plants.

MATERIAL AND METHODS.—All plants for this investigation were grown from seedlings supplied by the Intercontinental Rubber Company or from nursery stock[1] supplied by the U.S. Forest Service. The plants were planted in 4-mesh gravel, contained in 2-gallon glazed crocks, or in 1- or 2-gallon cans previously painted with a nontoxic asphaltum paint. All plants were supplied daily with Hoagland's nutrient solution (complete) and watered as needed with tap water. Plants were in part greenhouse-grown and in part grown outdoors (during the summer months).

Controlled temperature conditions were achieved in air-conditioned greenhouses, where the temperature was regulated to $\pm 1°$ F., and in a glass-roofed insulated box cooled with a 2-ton compressor through direct expansion coils and in which the temperature could be regulated to $\pm$ 1°.

Rubber determinations were made on material dried rapidly at 65° C. and ground in a Wiley mill. Two-gm. aliquots of the material were then extracted with absolute acetone for 16 hours. The acetone-soluble material is referred to as "resin." The acetone-insoluble residue was then extracted with benzene for 8 hours, the benzene removed, concentrated, and the solid residue dried and weighed. The benzene-soluble portion is referred to as "rubber." The rubber so obtained is impure, particularly when its concentration in the plant is under 0.5%. The determination is highly reproducible, however, and the results are of value from a comparative standpoint, even at low productive levels.

All plants were defoliated at the time of harvest and the leaves weighed separately from the stems and roots. Since the leaves contain but little rubber, the analyses given are based on the stem and root samples.

Experimentation

On January 2, 1942, a group of plants (grown from Intercontinental Rubber Company seedlings) 2½ months old from the time of transplanting from the seedling flat was selected for uniformity and randomized into four lots of twelve plants each. Of these groups, one was harvested as an initial control, one was placed in a controlled-temperature greenhouse at 80° F.[2] (day and night), one remained in the ordinary greenhouse, and one was removed to an open unshaded position outdoors. The average daily

[1] Owing to the critical shortage of guayule plants in the spring of 1942, the U.S. Forest Service was able to supply only cull nursery stock.

[2] All succeeding temperature references are Fahrenheit.

temperature in the greenhouse during the 2 months of the experiment was 72°, while the nightly minimum was 56°. Outdoors during the same period the average daily maximum temperature was 63°, while the average nightly minimum was 43°. At the expiration of 2 months all plants were harvested, composited in groups of two plants, dried, ground, and assayed for resin and rubber. The results of this experiment (G-25) are given in table 1. The plants increased markedly in dry weight during the 2 months under all conditions, although growth was greater in the greenhouses than outdoors. In the two greenhouses the percentage of rubber decreased significantly during the 2 months, although the total amount per plant increased. In the plants which remained outdoors, however, the percentage of rubber almost trebled over its initial value, rising to 1.5%, while the total amount per plant was double that of the larger plants which had remained in the greenhouse. These results in all probability were not due to the greater light intensity received by the outdoors plants, as can be seen from the experiment of table 2.

TABLE 1

GROWTH AND RUBBER FORMATION OF GREENHOUSE-GROWN GUAYULE PLANTS, JANUARY AND FEBRUARY, 1942. EXPERIMENT G-25. ANY COMPARISON ATTENDED BY 10° OF FREEDOM

Material	Dry wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
Initial plants	1.25±0.081	2.82±0.089	0.57±0.050	7
2 months at 80° F. (day and night)	8.33± .48	3.06± .090	0.36± .046*	30
2 months in ordinary greenhouse (ave. day max. 72°, night min. 55°)	7.25± .28	3.04± .162	0.40± .042*	29
2 months outdoors (ave. day max. 63°, night min. 43°)	3.83±0.28	4.07±0.043	1.52±0.066†	58

*Differs significantly from that of initial plants.
† Differs highly significantly from all others.

For this experiment (G-54), plants grown from cull nursery seedlings (spring, 1942, stock of the Intercontinental Rubber Company supplied by U.S. Forest Service) were used. These were grown outdoors in gravel contained in 1-gallon cans. On September 28, 100 plants selected for uniformity were randomized in five lots of twenty plants each. One lot was maintained in a greenhouse for 2 months and a second lot for 4 months. A third lot was maintained outdoors for 2 months in a lath-house, where the light intensity approximated that in the greenhouse. The fourth lot was maintained outdoors for 4 months, and the remaining lot served as the initial harvest. At the time of harvest each lot of twenty plants was harvested as ten composite groups of two plants each. During the first month of the experiment the heating system of the greenhouse failed to function, and the average nightly minimum temperature was approximately 50°. During the last 3 months of the experiment the average minimum nightly greenhouse temperature was 60°. As shown in table 2, the plants maintained outdoors steadily increased in rubber

concentration during the 4 months of the experiment; those in the greenhouse increased in percentage during the first 2 months but decreased during the second period. At the end of the experiment the outside plants had 4.5 times as high a rubber concentration as the plants maintained in the greenhouse.

A further experiment (G-26) also makes it appear unlikely that the results of tables 1 and 2 were due to lower light intensity in the greenhouse. For this experiment, use was made of plants which had been planted in the field in June, 1941. In a small portion of the field, thirty-six plants selected for uniformity were randomized in three lots of twelve plants each. Twelve plants were harvested as an initial control, twelve more were transplanted to 12-inch pots and removed to a greenhouse, while twelve were allowed to remain in the field. The twelve plants moved to the greenhouse were placed near a door which was kept open, so that the temperature approximated that outside. After 2 months the plants were analyzed (table 3). Although the transplanted plants made less growth than those which remained in the field, their increase in percentage of rubber was comparable with that of the field plants.

The experiments of tables 1, 2, and 3, taken together, suggest that temperature may influence increase in rubber concentration in guayule. The following experiments support such a supposition.

VARYING NIGHT TEMPERATURES.—For experiment G-39, a group of 100 greenhouse-grown plants (from seedlings supplied by the Intercontinental Rubber Company) selected for uniformity were divided into five lots. One lot was harvested immediately as initial control.

TABLE 2

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS, SEPTEMBER 28, 1942—FEBRUARY 1, 1943. EXPERIMENT G-54. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

MATERIAL	DRY WT. PER 2 PLANTS (GM.)		RESIN (%)	RUBBER (%)
	Stems and roots	Leaves		
Initial plants.....	3.66±0.24	5.22±0.22	4.35±0.154	0.53±0.034
In greenhouse 2 months	7.78± .43	8.39± .56	4.24± .114	1.03± .087
In greenhouse 4 months	11.23± .58	7.72± .27	3.48± .123	0.85± .062
Outdoors 2 months....	8.78± .56	7.42± .52	4.69± .075	2.69± .105
Outdoors 4 months....	10.14±0.44	4.77±0.29	4.92±0.102	3.79±0.159

Two lots were placed in a greenhouse maintained at 80°. Two lots were transferred to a similar greenhouse where the

TABLE 3

GROWTH AND RUBBER FORMATION OF FIELD-GROWN GUAYULE PLANTS, JANUARY AND FEBRUARY, 1942. EXPERIMENT G-26. ANY COMPARISON ATTENDED BY 10° OF FREEDOM

Material	Dry wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)
Initial plants.....	84.7±2.75	4.24±0.050	1.63±0.120
In field 2 months...	122.4±4.13	5.47± .121	4.50± .148
In greenhouse at outside temperature 2 months...	94.8±2.77	5.19±0.219	4.38±0.160

day temperature was maintained at 80° (8 hours) and the night temperature at 60° (16 hours). After 2 months one lot of twenty plants was harvested from

each temperature condition. There was no significant change in percentage rubber in either lot from the concentration found in the initial plants (table 4). The night temperature in the one greenhouse room was then changed to 55° and the experiment continued for another month, after which the remaining two lots were harvested. The rubber percentage in the plants maintained at 80° day and night was now significantly lower than that of the initial plants. In no treatment of this experiment was there a significant increase over that in the initial plants.

In a second experiment (G-62) in night temperatures of 80° and 55°, no significant change occurred in rubber percentage in plants left for 1 month at a constant temperature of 80°. Plants subjected to a night temperature of 55° yielded a small increase (table 4).

For all but one of the next six experiments the refrigerated box was used. For experiment G-42, sixty plants selected for uniformity (grown from cull nursery stock supplied by the U.S. Forest Service) were divided into three lots of twenty each. One lot was harvested as an initial sample. Another lot was removed to the greenhouse at a constant temperature of 80°, and the third lot was shifted daily so that it had an 8-hour day in the greenhouse at 80° and a 16-hour night in the refrigerated box at 50°. After 1 month, these two lots were harvested and analyzed. Table 5 shows that the rubber concentration decreased significantly in the plants maintained at 80° day and night but increased significantly in the plants which received a 50° night temperature. In a second experiment (G-43) at a 50° night temperature, somewhat similar results were obtained. Plants which received continuous temperature of 80° did not increase in rubber concentration, although they did not decrease as in the previous experiment.

TABLE 4

GROWTH AND RUBBER FORMATION OF GREENHOUSE-GROWN GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-39 AND G-62. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Exp. no.	Material	Dry wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
G-39a	1. Initial plants	7.04±0.17	3.11±0.075	0.60±0.059	42
	2. 2 months (80° day and night)	24.2 ±0.67	3.22± .172	0.45± .033	109
	3. 2 months (80° day, 60° night)	27.6 ±1.28	3.40± .074	0.47± .021	130
G-39b	4. Plants of 2 (39a) additional month at 80°	30.8 ±1.63	3.42± .092	0.37± .030*	114
	5. Plants of 3 (39a) additional month at 80° day, 55° night	37.4 ±1.45	3.72± .009	0.51± .032	191
G-62	1. Initial plants	2.22±0.140	3.41± .182	0.37± .022	8.2
	2. 1 month (80° day and night)	3.22±0.135	3.22± .049	0.38± .029	12.2
	3. 1 month (80° day, 55° night)	3.34±0.165	3.34±0.063	0.48±0.033†	16.0

* Significantly lower than that of initial plants.
† Significantly higher than that of initial plants.

Plants given nights at 50° doubled in rubber percentage during the month of the experiment. Somewhat less growth with the cool nights occurred in this experiment than in the preceding, possibly because during the first night the cold box thermostat failed, and the temperature dropped to near freezing. In experiment G-43 (table 5), one lot of twenty plants was maintained at 50° continuously at 80° did not increase in rubber concentration, whereas plants which received a night temperature of 45° trebled in percentage and increased in total rubber by 5.7 times during the month of the experiment. Plants maintained continuously at 45° did not grow and apparently decreased in rubber concentration. Although the plants which received 45° night temperature increased

TABLE 5

GROWTH AND RUBBER FORMATION OF GREENHOUSE-GROWN GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-42 AND G-43. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Exp. no.	Material	Dry. wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
G-42	Initial plants	8.58±0.38	4.06±0.176	0.43±0.036	37
	1 month (80° day and night)	14.4 ± .74	3.56± .173	0.23± .020*	33
	1 month (80° day, 50° night)	14.7 ± .48	3.83± .135	0.62± .050†	91
G-43	Initial plants	4.45± .25	3.95± .082	0.29± .012	13
	1 month (80° day and night)	9.91± .67	4.12± .028	0.28± .019	28
	1 month (80° day, 50° night)	6.44± .20	4.00± .149	0.61± .097†	39
	1 month (50° day and night)	4.35±0.22	4.10±0.254	0.35±0.017	15

* Significantly lower than that of initial plants.
† Highly significantly greater than initial plants of this experiment.

day and night. This lot made no growth and formed no rubber. The lack of growth may have been due in part to the difficulty of achieving a high light intensity within the box and at the same time regulating the temperature to 50° during the day. In part, however, it appears to be correlated with adaptation of the plant to the low day temperature. Plants removed from a warm greenhouse and placed in the 50° box during the day wilted severely for several days, even when adequately supplied with water.

The experiments of table 5 were continued with two experiments in which plants were switched daily between a day temperature of 80° and a night temperature of 45°. As can be seen from table 6 (experiment G-50), plants maintained in dry weight of stems and of roots as much as did the plants which received a night temperature of 80°, still the growth habits of the two lots were entirely different. Those plants which received a high night temperature showed rapid shoot growth and made abundant growth of new leaves during the month of the experiment; those which received a low night temperature made little new shoot growth, produced few new leaves, and had a dormant appearance in contrast to the first group.

In experiment G-52, use was made of the same temperatures as in G-50. The starting material for both experiments consisted of plants grown outdoors during the late summer. They were removed to the greenhouse on September

28 and on this date contained 1.00% rubber, in contrast to the 0.50% contained by the similar plants of G-50 when they were removed to the greenhouse on September 18. In experiment G-52, the rubber concentration of the plants maintained at 80° at night decreased markedly during the month of the experiment; those at 45° increased rapidly in percentage rubber during the same time.

TABLE 6

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-50 AND G-52. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

EXP. NO.	MATERIAL	DRY WT. PER 2 PLANTS (GM.)		RESIN (%)	RUBBER (%)	RUBBER PER 2 PLANTS (MG.)
		Stems and roots	Leaves			
G-50	Initial plants	5.43±0.33	6.46±0.42	4.33±0.082	0.50±0.030	27
	1 month (80° day and night)	11.6 ± .59	17.5 ±1.07	3.62± .172	0.43± .018*	50
	1 month (80° day, 45° night)	10.3 ± .34	11.3 ± .30	4.09± .099	1.49± .121†	153
	1 month (45° day and night)	5.03± .16	5.81± .31	3.38± .116	0.40± .021‡	20
G-52	Initial plants	5.62± .432	6.82± .430	4.39± .091	1.00± .112	56
	1 month (80° day and night)	11.40± .888	14.53±1.30	3.56± .092	0.71± .039‡	81
	1 month (80° day, 45° night)	10.94±1.02	11.26±1.40	3.72±0.092	2.01±0.142†	220

* Not significantly different from that of initial plants.
† Highly significantly greater than any other in this experiment.
‡ Significantly smaller than that of initial plants.

The two experiments of table 7 were similar to those of table 6, except that the night temperature was maintained at 40°. Results qualitatively similar to those with 45° night temperature were obtained, although the response in rubber formation was less striking.

Table 8 presents a summary of the 1–2 months' experiments with controlled day and night temperatures. (In addition to the ten experiments tabulated, eight further tests not here reported were carried out at less well-controlled low temperatures. Three other experiments will be summarized separately. The results were in all cases similar to those given.) In table 8, the change in percentage of rubber during the experiment for plants maintained at 80° is variable but small. In four cases the percentage decreased significantly and in six cases it did not; in no case was any significant increase noted. It is to be stressed that—even in the cases of apparent decrease in rubber percentage—the absolute amount of rubber present per plant increased (table 8).

In the three experiments with night temperatures of 60° and 55°, small or insignificant increases in concentration took place within the 1–2 months' period. At night temperatures of 50°, greater increases in rubber percentage were noted, and still greater were obtained when night temperatures were 45°. In the two experiments with night temperatures of 40°, the apparent response in rubber formation was smaller than with night temperatures of 45°. It may be that the optimum night temperature for rubber formation under the conditions used is approximately 45°.

TABLE 7

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-57 AND G-61. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Exp. no.	Material	Dry wt. per 2 plants (gm.)		Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
		Stems and roots	Leaves			
G-57	Initial plants	7.57±0.302	9.58±0.314	4.07±0.159	0.99±0.073	75
	1 month (80° day and night)	11.37± .460	13.41± .610	3.32± .061	0.77± .064*	88
	1 month (80° day, 40° night)	12.24± .519	13.46± .632	3.78± .136	1.65± .118†	202
	1 month (40° day and night)	8.78± .331	10.52± .452	3.54± .050	0.96± .069	84
G-61	Initial plants	2.93± .145	2.87± .175	4.11± .148	0.40± .034	12
	1 month (80° day and night)	4.80± .324	4.86± .310	3.54± .047	0.51± .038	24.5
	1 month (80° day, 40° night)	4.83±0.176	4.25±0.161	3.49±0.101	0.85±0.059†	41

* Significantly less than that of initial plants.

† Highly significantly greater than corresponding values for initial plants.

TABLE 8

SUMMARY OF EXPERIMENTS ON INFLUENCE OF VARYING NIGHT TEMPERATURES ON GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS

Exp. no.	Length of exp. (months)	Temperature (°F.)		Change in rubber (%)	Increase in rubber per 2 plants (mg.)	Grams rubber formed per 100 gm. increase in stem and root dry wt.
		Day	Night			
G-25	2	80	80	−0.21†	23	0.32
G-39a	2	80	80	−0.15	67	0.39
G-39b	1	80	80	−0.08	5	0.08
G-42	1	80	80	−0.20†	− 4	0.00
G-43	1	80	80	−0.01	15	0.27
G-50	1	80	80	−0.07	23	0.37
G-52	1	80	80	−0.29‡	25	0.43
G-57	1	80	80	−0.22†	13	0.34
G-61	1	80	80	+0.11	12	0.64
G-62	1	80	80	+0.01	4	0.40
G-39a	2	80	60	−0.13	88	0.43
G-39b	1	80	55	+0.04	61	0.62
G-62	1	80	55	+0.11†	8	0.71
G-42	1	80	50	+0.19‡	54	0.88
G-43	1	80	50	+0.32‡	26	1.31
G-50	1	80	45	+0.99‡	126	2.57
G-52	1	80	45	+1.01‡	164	3.09
G-57	1	80	40	+0.66‡	127	2.72
G-61	1	80	40	+0.45‡	29	1.53

† Significant at 5% level.

‡ Significant at 1% level.

Table 8 gives for each experiment the percentage of rubber in the dry weight deposited during the experimental period. At night temperatures of 80°, 60°, and 55° this percentage is low, and of the order of the percentage rubber found in greenhouse, nursery, or field plants during the first summer (in the Institute field at Arcadia). With night temperatures of 50°, however, rubber constituted about 1% of the added dry weight; and with night temperatures of 45°, it constituted 2.6–3.1% of the total added dry weight.

each condition (data used in table 6), and harvests were repeated after 2 and after 4 months. The data are given in table 9. Plants subjected to continuous temperature of 80° showed no significant change in rubber concentration over the 4-month period, although the absolute amount of rubber per plant increased 4.0 times. Plants subjected to the low night temperature increased steadily in rubber concentration and at the end of the 4-month period contained twelve times the amount present in the initial plants.

TABLE 9

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENT G-52. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

MATERIAL	80° DAY AND NIGHT				80° DAY AND 45° NIGHT			
	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)
	Stems and roots	Leaves			Stems and roots	Leaves		
Initial plants	2.81±0.216	3.41±0.215	4.39±0.091	1.00±0.112	2.81±0.261	3.41±0.215	4.39±0.091	1.00±0.112
1 month....	5.70± .444	7.26± .648	3.56± .092	0.71± .039	5.47± .509	5.63± .702	3.72± .092	2.01± .142
2 months...	6.38± .545	4.57± .347*	3.74± .102	1.04± .096	6.97± .775	4.30± .514	4.25± .080	3.21± .189
4 months...	13.06±0.607	11.92±0.714	3.65±0.089	0.85±0.069	7.60±0.60	5.34±0.412	4.59±0.087	4.18±0.138

* Reason for apparent decrease in leaf weight during this period not certainly known but may have been result of greenhouse fumigation.

Thus, under the conditions used these young plants appear to be nearly ten times as efficient formers of rubber with nights at 45° as they are with nights at 80°. Many other factors affect this efficiency, however, as will be next shown.

CONTROLLED TEMPERATURES OVER 4-MONTH PERIOD.—The plants used for this experiment (G-52) were of the cull nursery stock. They were grown in gravel contained in 1-gallon cans. On September 28, 1942, plants selected for uniformity were transferred either to the greenhouse having a constant temperature of 80° or to the similar greenhouse having an 8-hour day at 80° combined with a 16-hour night at 45°. After 1 month, sample plants were taken from

Table 10 gives certain data derived from those of table 9, including the ratio of grams of rubber deposited to total dry weight deposited. This quantity, which was used in table 8, expresses—in a formal way at least—the efficiency of the plant as a rubber producer during the experimental period. In the plants maintained continuously at 80°, 0.46 gm. of rubber was laid down per 100 gm. new dry weight during the first month and 0.68 gm. of rubber per 100 gm. new dry weight during the last 2 months. During the second month, however, it was higher, owing to unrecognized reasons—perhaps related to the unknown cause of the leaf-drop during the second month. The plants which received nights at 45°

increased in rubber-forming efficiency markedly as the experiment progressed. During the last 2 months, 15 gm. of rubber was accumulated per 100 gm. of new dry weight, or five times as much as during the first month. During the last 2 months the plants maintained at 45° night temperature formed approximately twenty-two times as much rubber per 100 gm. new dry weight as did the plants maintained at 80°.

OLDER PLANTS.—Parallel to the experiment of table 9, another was run with older plants (G-51). The two tests were run simultaneously and are comparable in every way, except for the nature of the plants used. The plants were grown from 1940 nursery stock of the Intercontinental Rubber Company planted in the field in June, 1941. The soil of the field (gravel subsoil) appeared to be unsuitable, and the plants made little growth as compared with similar plantings in other locations. In March, 1942, the plants were transferred to gravel in 2-gallon crocks and grown outdoors until September 28. They presented an appearance of typical field-grown plants, although of smaller size. When the plants were taken from the field in March they contained 4.88% rubber, and this declined to 2.58% on September 28. Total rubber per plant increased during the same time. On September 28, 100 plants were selected for uniformity and distributed as in the experiment of table 9, a portion being maintained in the greenhouse at a constant temperature of 80° and a further portion maintained at a day temperature of 80° combined with a night temperature of 45°. Harvests were made after 1, 2, and 4 months. The first two harvests were based on ten individual plants from each condition, while the final harvest was based on ten lots of two plants each from each condition. The results are presented in table 11. Growth of the plants during the first month was marked under the constant 80° conditions. Abundant new growth appeared, and the plants flowered profusely, although no significant increase in total dry weight occurred (table 11). Throughout the 4-month period the plants which received 45° nights maintained a dormant aspect, made little or no new shoot growth, formed no new leaves, and did not flower. They did, however, increase appreciably in dry weight. The plants which received 80° nights produced new leaves and flowers throughout the 4-month period.

TABLE 10

DATA ON AMOUNTS OF RUBBER PER PLANT, ETC., DERIVED FROM TABLE 9

MATERIAL	80° DAY AND NIGHT		80° DAY AND 45° NIGHT	
	Rubber per plant (mg.)	Grams rubber formed per 100 gm. of total increase in wt.	Rubber per plant (mg.)	Grams rubber formed per 100 gm. of total increase in wt.
Initial plants	28	.....	28	.....
1 month....	40	0.43	110	3.1
2 months...	66	1.06	224	4.7
4 months...	111	0.81	318	6.0
2 months (last).....	.....	0.67	.....	14.9

With these plants, as with those of table 9, there was considerable difference in rubber formation between the two temperatures. Plants kept continuously at 80° did not change significantly in rubber concentration during the 4-month period, whereas those which received 45° nights increased steadily and more than doubled their original concentration. In fact, these latter plants had a final con-

centration as high as that of similar plants left outdoors during the same 4-month period (table 11).

It is not desired to stress the results of this experiment, since growth of the plants was relatively small during the experimental period (table 11). Growth of the same plants was rapid during the summer, and the subsequent lesser growth may have been due to a pot-bound condition. The conclusions drawn are therefore strictly applicable only to these particular plants.

TABLE 11

GROWTH AND RUBBER FORMATION OF OLDER GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENT G-51. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

MATERIAL	80° DAY AND 80° NIGHT				80° DAY AND 45° NIGHT			
	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)
	Stems and roots	Leaves			Stems and roots	Leaves		
Initial plants	72.2±4.73	42.8±1.91	4.49±0.093	2.58±0.233	72.2±4.73	42.8±1.91	4.49±0.093	2.58±0.233
1 month	69.8±3.46	48.3±1.72	4.03± .084	2.38± .157	80.6±4.58	41.9±1.73	4.71± .177	3.21± .281
2 months	81.9±4.27	54.2±2.56	4.22± .145	2.56± .167	95.5±3.52	45.2±1.50	4.84± .092	4.18± .211
4 months	83.6±2.56	46.2±2.50	4.37±0.063	2.67±0.195	90.2±3.00	37.2±1.50	4.94± .116	5.75± .107
4 months outdoors*					78.5±4.06	12.7±0.83	5.42±0.103	5.50±0.212

* Many leaves dislodged by heavy rain.

TABLE 12

DATA ON AMOUNTS OF RUBBER PER PLANT, ETC., DERIVED FROM TABLE 11

MATERIAL	80° DAY AND 80° NIGHT		80° DAY AND 45° NIGHT	
	Rubber per plant (gm.)	Grams rubber formed per 100 gm. total increase in dry wt.	Rubber per plant (gm.)	Grams rubber formed per 100 gm. total increase in dry wt.
Initial plants	1.86	.....	1.86	.....
1 month	1.67	.....	2.58	8.7
2 months	2.09	2.37	3.99	9.1
4 months	2.23	3.24	5.20	18.5

Table 12 presents data derived from table 11 as to total rubber per plant and as to rubber deposited per unit of added dry weight. Comparison of tables 10 and 12 shows that: night temperature of 45° induced more rubber formation than night temperature of 80°, both with young and with older plants; with both kinds of plants, response to the lower night temperature was more striking over a 4-month than over a 1-month period; and under both temperature conditions the older plants were markedly more efficient rubber producers than the young ones.

It would appear from the preceding experiments that night temperatures may be of importance in regulating rubber formation in guayule. That other factors, such as nutritional status, water supply, etc., also influence rubber accumulation may be assumed. The relation of these several factors and the relative significance of each are matters for further investigation.

Summary

1. Young greenhouse-grown plants of guayule showed marked difference in rubber formation according to the temperature conditions to which the plants were subjected.

2. Plants grown in the greenhouse or outdoors in the summer contained in general 0.50% or less rubber. When such

plants were transferred to a greenhouse at a constant temperature of 80° F., rubber concentration either remained at the initial low level or dropped slightly.

3. Similar plants subjected to an 8-hour day at 80° and a 16-hour night at 45° or 40° increased in percentage of rubber. This increase amounted to 0.7–1.0% over a period of 1 month.

4. Night temperatures of 50° appeared to be less effective than night temperatures of 40°–45° in causing increase of rubber percentage. Night temperatures of 55°–60° were ineffective.

5. Young plants continued to respond to night temperatures of 45° with increased rubber production over a period of 4 months.

6. Older plants, which had already been subjected to one winter in the field, responded to low night temperatures in a manner qualitatively similar to that of young plants. The older plants, however, were more effective in rubber accumulation than the young plants, both at high and at low night temperatures.

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NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF LEPIDIUM DRABA[1]

JOHN C. FRAZIER

Introduction

A series of studies of growth habits of noxious perennial weeds of central United States was undertaken at Manhattan, Kansas, in 1937 by the Kansas Agricultural Experiment Station. A study (1) of the nature and rate of development of the root system of field bindweed established the scope and methods of procedure. The second of these investigations concerns *Lepidium draba* L.,[2] to which several common names have been applied, including hoary cress, whitetop, whiteweed, and perennial peppergrass. It is a noxious weed of potentially equal or greater significance than field bindweed in this area.

Environmental conditions and methods

SOIL DATA.—All plants[3] considered in this paper were taken from soil, the nature of which is described in the following profile description supplied by Dr. J. C. HIDE of Kansas Agricultural Experiment Station:

Depth 0–8 inches.—A dark grayish brown silt loam to silty clay loam with weakly developed platy structure, except in the lower 2 inches which has a slightly granular nature.

Depth 8–14 inches.—A dark brown loamy clay; moderately compact. Angular cleavage with only slight, if any, tendency to columnar structure. Aggregates relatively fine, about $\frac{1}{8}$ inch. A few small limestone fragments.

Depth 14–22 inches.—A dark yellowish brown loamy clay with fairly well-developed fragmental to blocky structure. Little tendency to form columns. Moderately friable.

Depth 22–60 inches.—A yellowish brown loamy clay. Blocky aggregates show little tendency to form columns. Small fragments of limestone abundant but less numerous below 36–38 inches. Below 32 inches, lime has been deposited in root channels.

The soil was a fairly typical Geary silt loam, except that it lacked the reddish brown color distinctive of that soil type. No layer of this profile appears to be of such nature as to impede seriously the development of the roots of deep-rooting plants.

METEOROLOGICAL DATA.—The monthly and annual temperature and precipitation for the first 10 months of 1941 compared with the long-time average of these two factors are given in table 1. The summer of 1941 had temperatures of 100° F. or higher for 15 days, as compared with 38 such days in 1937 and 60 days in 1936. The average number of days at this temperature for the 50 years (1889–1938) is 15. The monthly and annual mean temperatures were higher than those for the 85-year period, 1856–1940, except for the monthly means of March and June. The average monthly precipitation for the first 10 months of 1941 was 3.22 inches, as compared with the monthly average 2.86 for these months during the 83-year period (1858–1940).

METHODS.—The plants were grown on a small plot of land free of both noxious

[1] Contribution no. 451, Department of Botany, Kansas Agricultural Experiment Station.

[2] The great variations in the degree of veining and in the shape of the capsule within our individual plants correspond to the findings of HITCHCOCK (2); hence varieties under the species are not recognized.

[3] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.

weeds and sodium chlorate. It adjoined that described previously (1). The soil is a fairly typical Geary silt loam representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

Plantings were made on April 21, 1941. Twenty-five seeds, which had been kept moist at 34° F. for 41 days, were placed $\frac{5}{16}$ inch below the surface at each of twenty-two points which were so spaced that seven of the plantings were 14 feet distant from any other, and the remainder were separated by 5–9 feet. Emergence of seedlings began on April 26, and on May 1 at least one seedling emerged at eighteen of the twenty-two points. All previously emerging seedlings were removed, as were those emerging after this date; consequently, all plants considered in this study emerged on May 1. The area was kept free of other vegetation, so that the only competition for water and nutrients, if any existed, was among the plants of hoary cress.

Root systems were excavated at weekly intervals for the first 7 weeks, and also 9, 12, 26, and 75 weeks after emergence, by modification of the trenching method developed by WEAVER (6) as described by FRAZIER (1).

Effort was made to keep the root system as one entity; if one broke, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, and the relationship of parts noted as the root system was excavated, so that the plant as illustrated occupied the same relative relationship one part to another as it did in the soil, except that in the figures all lateral roots are arranged in one plane. While as many of the finer roots were obtained as possible, it is not contended that a large proportion was secured.

Observations

The rate of plant development under known soil and climatic conditions was observed by means of seven plants excavated weekly between May 8 and June 19, and plants 8, 9, and 10 excavated July 3, 24, and in late October, 1941, respectively. An eleventh plant was excavated in mid-October, 1942. Figures 1–5, inclusive, show the vertical penetration and figures 3–5 the radial spread of representative plants. Data of the eleven excavations are given in table 2.

TABLE 1

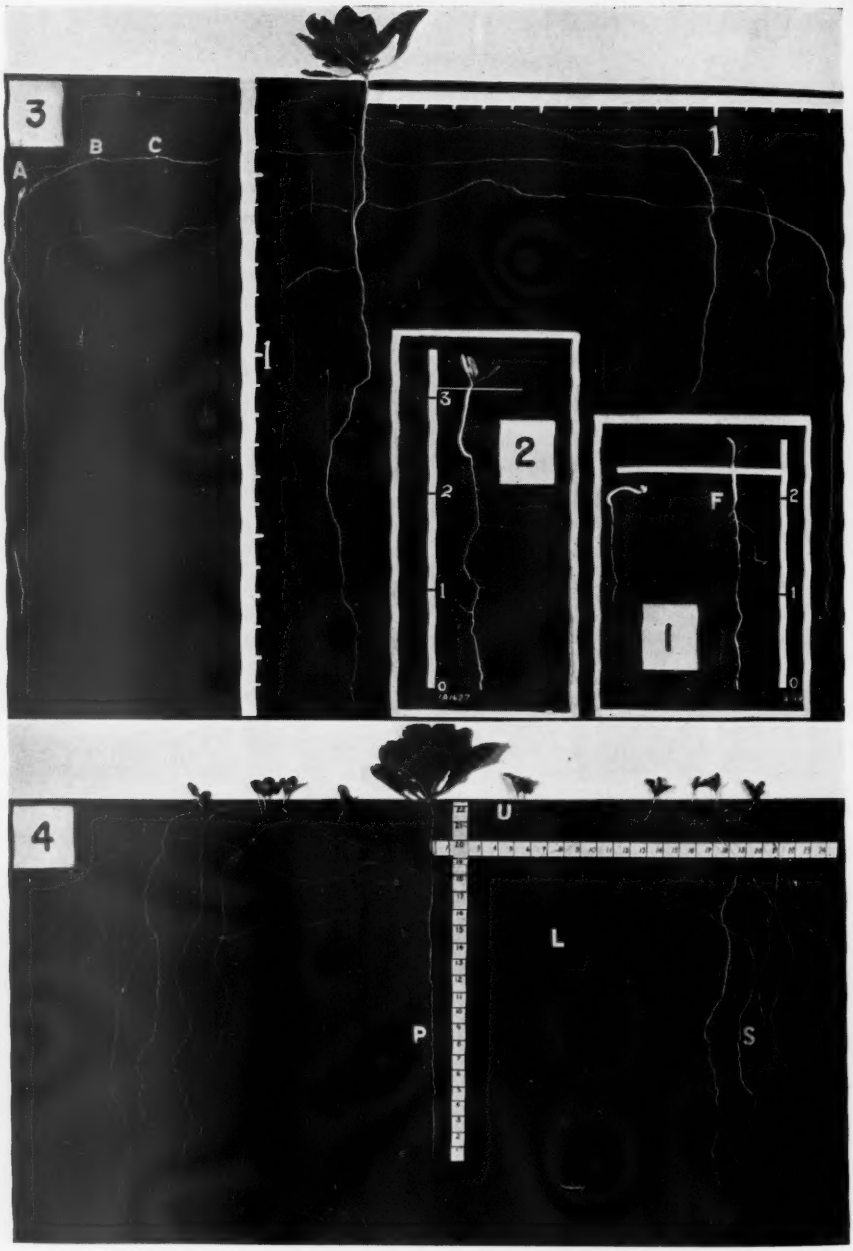
MONTHLY AND ANNUAL TEMPERATURES (IN °F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1941

Month	Temperature		Precipitation	
	Mean*	85-year average (1856–1940)†	Total*	83-year average (1858–1940)†
Jan.	32.2	27.0	2.08	0.75
Feb.	33.1	31.5	0.28	1.14
Mar.	40.6	42.5	1.23	1.48
Apr.	58.5	54.5	1.81	2.67
May	69.6	64.6	2.36	4.31
June	74.0	74.5	3.80	4.50
July	81.4	79.5	1.29	4.25
Aug.	79.8	77.5	3.00	3.89
Sept.	71.9	69.3	4.85	3.44
Oct.	59.2	56.5	11.50	2.16
Annual mean or total	57.0	54.2	33.93	30.97

* Meteorological data obtained from U.S. Weather Bureau, Kansas Section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

By the seventh week after emergence, the general plan of the root system was beginning to be established. The plant taken the twelfth week after emergence (fig. 4) showed the gross morphological nature of the root system particularly well. A taproot rapidly penetrated directly down from the germinating seed.



FIGS. 1–4.—Development of plants of hoary cress. Fig. 1, seedling at left had not emerged, seedling at right one week after emergence; *F*, root-stem transition zone. Fig. 2, seedling 2 weeks after emergence showing first true leaves. Fig. 3, young plant 9 weeks after emergence: *B*, *C*, root-borne stem buds; *A*, bud giving rise to rhizome; secondary vertical roots have grown downward 15–16 inches. Fig. 4, complete plant 12 weeks after emergence (not all primary vertical root obtained): *P*, primary vertical root; *L*, lateral root of first order; *S*, secondary vertical of first order; *U*, rhizome arising from lateral root. Figs. 1, 2, 4, scale in inches; fig. 3, scale in feet and inches.

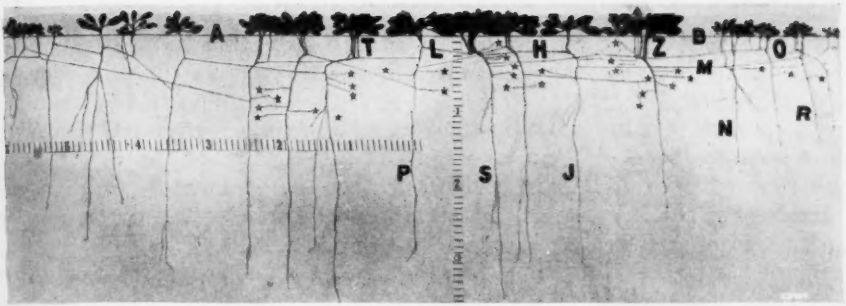


FIG. 5.—Approximately 60% of growth attained by one plant of hoary cress 6½-months after emergence, arranged to show place relationship of parts essentially as they were in the soil. Stars indicate points from which lateral roots have been removed for clarity. *A–B* is ground line. Lateral roots to left of primary vertical developed under sloping ground line, which causes them to appear to grow upward from point of origin when arranged to a horizontal ground line. *P*, primary vertical root; *L*, permanent lateral of first order; *S*, secondary vertical of first order; *H*, permanent lateral of second order; *J*, secondary vertical of second order; *M*, permanent lateral of third order; *N*, secondary vertical of third order; *O*, permanent lateral of fourth order; *R*, secondary vertical of fourth order. Scale in feet and inches.

TABLE 2

RATE AND NATURE OF DEVELOPMENT,* AFTER SEEDLING EMERGENCE, OF ROOTS OF HOARY CRESS PLANTS

Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1.....	2¼	.....	Long, somewhat linear cotyledons (fig. 1)
2.....	3⅛	1⅛	First true leaves (fig. 2)
3.....	6¾	2	Additional true leaves
4.....	10¾	3⅞	Older leaves becoming characteristically shaped
5.....	14¾	6¼	Several new leaves; forming loose rosette
6.....	18⅛	10	Cotyledons lost
7.....	19½	15¾	Lateral roots growing downward at tip; first root-borne stem buds on lateral roots, 13–14 inches from primary vertical
9.....	21¼	16½	Well-developed root-borne stem buds on primary permanent lateral and near juncture of that root and secondary vertical roots, one of which is developing into a rhizome; vertical roots have grown downward 15–16 inches (fig. 3)
12.....	22+	24½	Root-borne stem buds have formed rhizomes, most of which have emerged and developed shoots. Not all of primary vertical root excavated (fig. 4)
26.....	37	73½	By now the root development included permanent lateral and secondary verticals through fifth order (fig. 5)
75 (1942)..	11¼ ft.	At least 10–12 ft.	Secondary vertical roots apparently penetrated deeper than primary verticals and were larger in diameter, particularly in first 12–24 inches below bend where lateral root becomes secondary vertical

* First flowering of these plantings occurred June 18, 1942.

It was a primary root in order of development and vertical in position; hence, designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably located, grew extensively and became permanent parts of the root system. They are designated permanent lateral roots (fig. 4*L*). These grew horizontally 12–26 inches and then turned down in a broad sweeping bend and were from that point vertical taproots, designated secondary vertical roots[4] (fig. 4*S*). The permanent lateral roots of any order, which are usually smallest in diameter at their origin, were always in the surface foot, more commonly in the upper 7 inches of the soil. The lateral-vertical roots of the first order gave rise to branch roots over any part of the horizontal or vertical extent. Most of these were small feeding roots. However, at or just below the bend where the primary permanent laterals become secondary vertical roots, permanent laterals of the second order developed (fig. 5*H*). These extended horizontally 7–20 inches from the point of origin, the general direction of growth being away from the primary vertical root, and turned down to form secondary vertical roots of the second order (fig. 5*J*). This development was repeated many times during the growing season to give rise to lateral-verticals of the third, fourth, and even higher orders. It is by such series of permanent lateral roots that horizontal spreading of the plant is accomplished. If more than one permanent lateral arose from the place of origin of such roots of the second or higher order, they radiated from the point of origin; but, in general, all maintained growth away from the primary vertical root. The result was that the plant occupied a higher proportion of the ever-increasing area, somewhat circular in shape, than it would have otherwise.

All shoot development, except that from the plumule, was derived from root-borne stem buds. These developed on any part of the permanent root system (that is, the permanent laterals and the two types of vertical roots). Twelve weeks after emergence, a number of these buds had been formed on or near the juncture between the permanent lateral and the secondary vertical roots, as well as a few along the permanent lateral roots themselves, and most of these had produced rhizomes which on emergence had produced shoots (fig. 4). The location of most extensive shoot development from root-borne stem buds was at or near the bend between the lateral root of any order and its vertical phase (fig. 5*T*, *Z*). Infrequently, shoots not subtended by a vertical root were found along the permanent laterals (fig. 4*U*). Regardless of where these root-borne stem buds formed, they gave rise to rhizomes unless the buds were borne at the ground line, in which case they gave rise to leafy shoots.

Fourteen weeks later, or 26 weeks after the plant had emerged as a seedling, the primary vertical root had penetrated 37 inches vertically and had given rise to nine horizontal roots which were arranged radially around the primary vertical root. All nine of these permanent laterals had developed secondary vertical roots. Seven of the secondary verticals of the first order had given rise to lateral-secondary vertical roots of the second and higher orders. There were seventeen regions of shoot development on the nine permanent lateral roots. The

[4] The horizontal and vertical phases of these roots are included in the term lateral-vertical root. The vertical phase is considered to begin where the slender lateral root enlarges markedly.

shoot growth of these seventeen regions came from forty-one rhizomes, each of which arose from a root-borne stem bud. The growth attained 26 weeks after emergence was so carefully removed that practically all the plant was recovered. Approximately 60% of the growth constituting the plant was arranged to give the place relationship of these parts essentially as they were in the soil (fig. 5).

Discussion

The rate of growth of plants of hoary cress from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential under this favorable noncompetitive situation.

SIMONDS (5) reported no structural differences between the horizontal and perpendicular roots of this plant and found that both produce adventitious buds which develop into shoots.

No reason was determined why certain of the branch roots develop more extensively to become permanent lateral roots. It appears probable that this is a result of their more favorable location. KENNEDY and CRAFTS (3) gave this explanation for the development of permanent lateral roots in field bindweed.

The observations of PEITERSEN and BURDICK (4) and SIMONDS (5) on the general nature of the vertical and horizontal portions of the root system of hoary cress agree with the findings of this study. The former designated the horizontal roots as root stocks, which is misleading, in that this term is commonly used for rhizomes (underground stems).

Summary

1. Hoary cress plants, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions, and not subject to competition, were studied at various ages, from the seedling stage through 75 weeks of growth, to determine the nature and rate of development.

2. The root system consisted of the original root (primary vertical) and one to many permanent laterals, the vertical extensions of which were designated secondary vertical roots.

3. The plants spread horizontally by means of series of permanent lateral roots. The primary permanent laterals arise on the primary vertical root. Unless injury or too severe competition prevents, successive orders of permanent laterals arise at or near the bend where a permanent lateral of the preceding order turns down to become a vertical root.

4. The plants spread radially $6\frac{1}{8}$ feet in one growing season and $11\frac{1}{4}$ feet by the end of the second season, at which time many verticals had reached a depth of 10–12 feet.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which form either leafy shoots directly (if at the ground line) or rhizomes (if below ground) which in turn give rise to leafy shoots. These arose in greatest abundance at or near the bend separating the lateral root of any order from its vertical phase. The shoot development of old plants is wholly from root-borne buds.

6. The type of development is similar to that of field bindweed. The rate of development of hoary cress does not appear to be as rapid as that of field bindweed during the first 75 weeks of growth.

DEPARTMENT OF BOTANY
KANSAS AGRICULTURAL EXPERIMENT STATION
MANHATTAN, KANSAS

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GERMINATION OF THE NUTS OF THE TUNG TREE AS AFFECTED BY PENETRANTS, SUBSTRATA, DEPTH OF PLANTING AND STORAGE CONDITIONS

C. B. SHEAR[1] AND H. L. CRANE[2]

Introduction

A method which would materially shorten the period required for and produce more uniform germination of tung (*Aleurites fordii* Hemsl.) nuts would alleviate difficulties experienced in nursery practice. The accepted practice is to plant the nuts in February or March. About 60 days are required for germination, but many seeds do not germinate until the following year. Emergence occurs when there is likelihood of injury to the seedlings by high temperatures. Quick, uniform germination of the seeds would produce trees of less variable size and suitable for budding earlier in the summer than normally is possible with present methods.

SHARPE and MERRILL[3] found that seeds that were hulled and stratified consistently germinated earlier than those subjected to other treatment, apparently mainly due to the intake of water during stratification.

At the time the present experiments were planned, the beneficial effects of stratification had not been reported, but under aseptic conditions the kernels of shelled nuts showed that initiation of germination was possible within 5 days. This indicated that any delay was due to inability of the seeds readily to absorb water through the thin but hard shell of the nut. Experiments were planned to determine: (*a*) whether it was possible to increase the rate of water absorption through the use of surface-tension-reducing chemicals; (*b*) the effect on germination of various substrata differing in their water-holding and aeration capacities; (*c*) the effect of planting depths; and (*d*) the effect of various periods of storage at different temperatures.

[1] Junior Physiologist and [2] Principal Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering; Agricultural Research Administration, U.S. Department of Agriculture.

[3] SHARPE, R. H., and MERRILL, SAMUEL, JR., Effects of stratification and time of planting on germination of tung seed. Proc. Amer. Soc. Hort. Sci. 40: 286–291. 1942.

Experimental results

EFFECT OF PENETRANTS

The penetrants used were morpholine and tergetol, both products of the Union Carbide and Carbon Corporation of New York. To test their effect on germination, lots of 100 open-pollinated seeds from a single tree, after careful cleansing, were treated as follows: morpholine 0.25, 0.5, and 1.0% for 12, 24, and 48 hours; tergetol 0.1, 0.2, and 0.4% for 12, 24, and 48 hours; water for 12, 24, and 48 hours; and untreated.

The nuts were placed in glazed crocks and covered with the solutions of morpholine or tergetol, or with water, at such times that the 12-, 24-, and 48-hour treatments would all be ready for removal and planting at the same time. The temperatures of the solutions and water varied from 24° to 27° C. Upon removal from the solutions, the lots of seeds were rinsed with tap water and planted in the greenhouse in a bed of well-rotted sawdust, the treatments being arranged in randomized replications of twenty seeds each. The planting was made on March 13, 1941; and emergence counts were made daily, with a few exceptions, after April 1—when the first seedlings appeared.

MORPHOLINE.—The data for cumulative emergence on successive days after

planting for the 48-hour treatments are graphed in figure 1. Chi square values for a number of pertinent comparisons, also for the 48-hour treatments, are presented in table 1. A marked increase in rate of emergence over unsoaked seeds

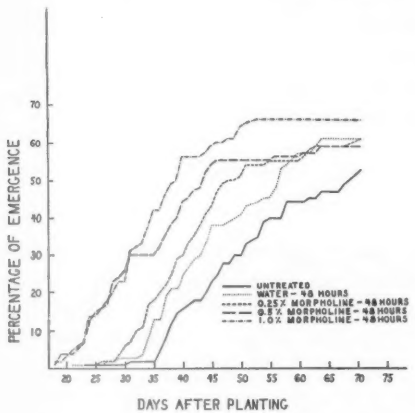

FIG. 1.—Effect of treatment with water and morpholine at different concentrations on percentage of emergence of tung seedlings.

resulted when the seeds were soaked in water for 48 hours; similar increases resulted from soaking for 12 or 24 hours. Morpholine at a concentration of 0.25% for 48 hours increased the rate significantly over that obtained with the same period of treatment with water, but after 12 or 24 hours at this concentration the rate of emergence was not significantly increased over that resulting from an equal period of soaking in water. When the concentration was increased to 0.5%, treatment for 12 hours was as effective as was the 48-hour treatment in the 0.25% solution. Soaking for 24 hours significantly increased the rate over the 12-hour treatment, and the 48-hour treatment was significantly increased over the 24-hour one.

Increasing the strength of the solution to 1.0% and soaking for 12 hours showed no advantage over the 0.5% treatment for the same period, but at 1.0% for 24 hours the results were equivalent to those obtained at 0.5% for 48 hours. However, when a 1.0% solution was used for 48 hours, the rate of emergence was increased significantly over that resulting from treatment with the same concentration for 24 hours. Comparison of the curves for these treatments in figure 1 shows that soaking the seed in 1.0% morpholine for 48 hours resulted in comparable percentages of emergence 12–18 days ahead of seed soaked for the same period in water, and 16–30 days ahead of untreated seed. There was no indication of any toxic effect from any of the morpholine treatments used.

There is also a definite tendency for decrease in rate of emergence to be associated with decrease in the final percentage of germination. At the termination of the experiment, the oil in the endosperm of many of the ungerminated seeds had solidified. This condition is probably an important factor in decreased viability of seed whose germination has been delayed.

TABLE 1

CHI SQUARE VALUES FOR COMPARISONS OF RATES OF EMERGENCE OF TUNG SEEDLINGS

Contrast of treatments (each 48 hours)	df	Chi square*
Untreated vs. H_2O	42	8.6
Untreated vs. 0.25% morpholine	40	49.6
Untreated vs. 0.5% morpholine	42	279.8
Untreated vs. 1.0% morpholine	42	226.3
H_2O vs. 0.25% morpholine	40	18.8
H_2O vs. 0.5% morpholine	43	167.5
H_2O vs. 1.0% morpholine	43	162.7

*Goulden, C. H., Methods of statistical analysis. John Wiley and Sons, New York. 1939.

TERGETOL.—All the tergetol treatments, with the exception of 0.1% for 12 and for 24 hours, were toxic to such an

extent that germination was reduced to 0–10%. Tergetol at a concentration of 0.1% for 24 hours was as effective in hastening emergence as was the 48-hour treatment with 0.5% morpholine, indicating the former a much more powerful penetrant. However, the beneficial effects obtained with tergetol are of doubtful value in practical application, because of the risk of toxic effects.

As the concentration of morpholine increased, the rate of emergence was not in proportion to the increase in moisture content of the kernel, which was approximately the same in seeds treated with 1.0% morpholine as in those treated with 0.5% for the same periods. The rate of emergence of the former was markedly higher than that of the latter, indicating that morpholine has some effect on germination beyond the increase in moisture content at the time of planting. There are two possible explanations for this effect: (*a*) At the higher concentration, morpholine, owing to its marked solvent properties, may increase the permeability of the shell to such an extent that, after planting, the rate of supply of water and of oxygen to the germinating embryo is greater than for seed treated with lower concentrations. (*b*) Morpholine, structurally related to ethylene, which has been found to stimulate proteolytic enzyme activity, may stimulate germination in this or some other way.

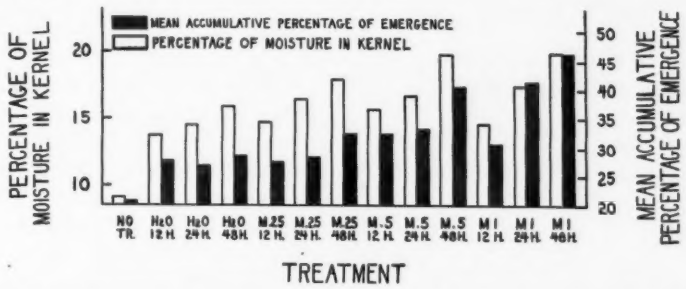

FIG. 2.—Relation of percentage of moisture in kernel at time of planting to mean cumulative percentage of emergence of seedlings. Mean cumulative percentage of emergence obtained by dividing the total percentage of emergence from the eighteenth to the seventy-first day after planting by the number of observations included in this total.

EFFECT OF MOISTURE CONTENT OF KERNELS

Samples of twenty nuts each, in duplicate, were treated with morpholine in the same concentrations and for the same periods as those used in the germination tests. After soaking, the shells were removed, the weights of the kernels obtained, and they were then dried for 20 hours at 100° C., again weighed, and moisture percentages calculated.

In figure 2 the percentage of moisture in the kernels is plotted against the mean cumulative percentage of emergence for the same treatments. There is a positive correlation between the rate of emergence and the moisture content of the kernel at the time of planting. The coefficient of correlation between these two measurements is 0.869, with a standard error of 0.0738.

EFFECT OF VARYING SUBSTRATA

It has been our practice to use well-rotted sawdust as a substratum for germinating seed. In 1942 it was necessary to use fresh sawdust—containing con-

siderable soil—for part of the planting, as the source of rotted sawdust had been depleted. The rate and final percentage of germination in the fresh sawdust were so superior that an experiment was set up to determine the effects of different substrata on germination.

Six media, varying widely in respect to air- and water-holding capacities, were selected: (*a*) sphagnum moss; (*b*) Berkeley Springs "F" grade glass sand; (*c*) Berkeley Springs medium-grade glass sand; (*d*) a mixture of 64 parts by weight of fresh sawdust plus 36 parts of a one-to-one mixture of the medium and 8-mesh glass sand; (*e*) a mixture of 16 parts by weight of cinders screened through a 4-mesh screen plus one part of screened sphagnum moss; and (*f*) well-rotted sawdust.

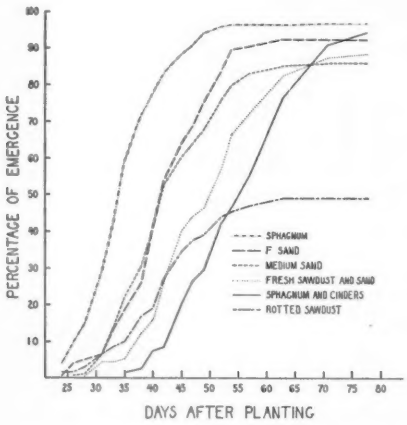

FIG. 3.—Effect of substrata on percentage of emergence of seedlings.

A lot of 200 open-pollinated seeds from one tree was planted at a depth of $\frac{3}{4}$ inch in each of these substrata, all seeds having previously been treated with 1.0% morpholine for 48 hours. The planting was made on January 30, 1942, and emergence counts were taken at 2- to 3-day intervals after February 23, when the first seedlings appeared.

Interpretation of the data for cumulative emergence in the different substrata (fig. 3) is complicated by the fact that the bench in which the experiment was planted abutted the west wall of the greenhouse, thus subjecting the sphagnum and cinder treatment to lower temperatures than the others. This condition probably contributed to the delayed germination of this treatment, and this temperature effect may have influenced to a lesser extent the germination in the fresh sawdust and sand treatment, which was second from the wall. Reliable evaluation of the effects of this condition being impossible, it cannot be considered in the comparison of results.

The outstanding features of the data in figure 3 are the results in the sphagnum moss. Both rate and final percentage of emergence were greatly increased in this medium over those in the other media, the chi square value for the comparison of the rate of emergence in this substratum with that resulting in rotted sawdust being 101.5, with 17 degrees of freedom. The fresh sawdust and sand mixture produced significantly better results than did the rotted sawdust or the sphagnum and cinder mixture, and both sands proved to be even more favorable media, the F sand showing a slight advantage over the medium sand.

EFFECT OF PLANTING DEPTH

Seed was taken from the same lot as that used in the substratum experiment, treated with 1.0% morpholine for 48 hours, and planted in fresh sawdust at three depths—$\frac{1}{2}$, 1, and 2 inches. Each treatment consisted of five replications of twenty seeds each. Planting was done on January 30, 1942, and emergence counts were taken at 2- to 3-day intervals

after the first seedlings appeared, on March 6.

At a depth of ½ inch, emergence commenced (fig. 4) at about the same rate as at 1 inch, but after 2 weeks this rate began to fall off, the final percentage of emergence attained by this lot being only 69% as compared with 93% for the seed planted at a depth of 1 inch. When seed was planted 2 inches deep the rate of emergence was greatly retarded, and the final percentage was 47%.

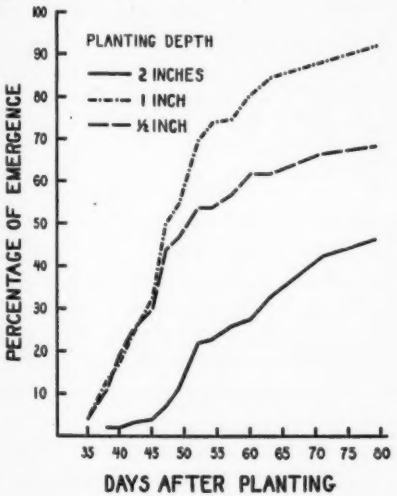

FIG. 4.—Effect of depth of planting on percentage of emergence of seedlings.

EFFECT OF STORAGE TEMPERATURE

The remainder of the seed from which the samples used in the morpholine and tergetol treatments were taken was divided into three lots, each being placed in storage at different temperatures on March 11, 1941. Prior to this date this seed had been stored (from December, 1940, to the first week of March, 1941) in the loft of a garage at the U.S. Field Laboratory for Tung Investigations, Bogalusa, Louisiana. One of the three lots was stored in a refrigerated room kept at approximately 7° C. The second lot was kept in the laboratory, where the temperature during this period was 23°–32° C. The third lot was stored in the attic of the laboratory building at Beltsville, where the temperature varied from

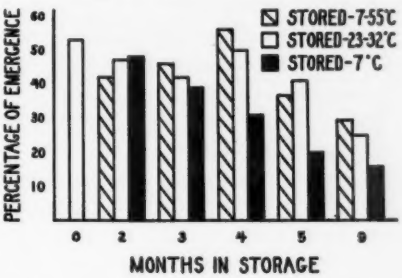

FIG. 5.—Effect of storage temperature and length of storage on percentage of emergence of seedlings.

about 7° to 55° C. Samples of 100 seeds were removed from each of these lots after storage for 2, 3, 4, 5, and 9 months and planted in rotted sawdust in a greenhouse bench. Planting was done in five randomized replications of twenty seeds each.

After 2 months' storage there was a decrease in the viability in all treatments (fig. 5), the greatest drop being in the sample taken from the lot stored at the highest temperatures. At 3 months, germination continued to decrease in the seed from the refrigerator and laboratory but increased in that from the attic. After 4 months in storage, both the lot from the attic and that from the laboratory showed increased germination. The refrigerated seed continued to lose viability, however, but that from the attic and laboratory continued to maintain an advantage over that from the refrigerator.

Discussion

The data presented in this paper substantiate the theory that the delayed germination of tung nuts under ordinary planting conditions is due primarily to

their slow rate of water absorption. They also corroborate the finding of SHARPE and MERRILL that the greater water intake during the period of stratification is responsible for the beneficial effect of this treatment.

The results of the substrata and depth-of-planting experiments not only further emphasize the importance of adequate water supply in hastening germination, but also indicate that, even when the moisture content of the seed is high at the time of planting and planting is done in a substratum of high water-holding capacity, some other factor has an important bearing on the rate and final percentage of germination. The indications are that this factor is aeration.

Although determinations of the relative water- and air-holding capacities of all the different media were not made, there are outstanding differences as regards these characteristics which are easily recognizable. Sphagnum, the substratum in which best results were obtained, undoubtedly has the greatest water-holding capacity of all the media used; at the same time it probably presents the most favorable conditions of aeration.

Determinations made in connection with the use of medium sand and F sand in nutritional experiments indicate that the F sand has only one-half the water-holding capacity of the medium sand but has sixteen times the air-holding capacity. Both of these media would be more subject to drying than the sphagnum, but frequent watering during the course of the experiment prevented moisture from becoming a critical factor in these substrata. The slight difference between the rates of germination in these two media is in favor of the coarser sand.

Even the addition of sand to the fresh sawdust evidently did not increase the porosity enough to supply adequate aeration for optimum germination, and the same condition was probably responsible for the slow rate of emergence in the screened sphagnum and cinder mixture.

The very slow rate of emergence and low final percentage of germination in the rotted sawdust undoubtedly are due to the tendency of this medium to pack and practically exclude air.

In the depth-of-planting experiment, the poor results obtained at a depth of ½ inch are probably due to drying of the seed between applications of water, but the drop in rate and final percentage of germination resulting from planting at a depth of 2 inches seems to be further evidence of the detrimental effects of lack of aeration.

Summary

1. Morpholine was highly effective in increasing the rate of germination of tung nuts. Soaking in 1.0% for 48 hours attained practically the same percentage of emergence 12–18 days prior to that of nuts soaked for the same period in water, and they were 16–30 days ahead of untreated seed.

2. Seed planted in sphagnum reached the same percentage level of seedling emergence 29 days ahead of that planted in rotted sawdust, 21 days ahead of that planted in a mixture of sphagnum and cinders, 16 days ahead of that planted in a mixture of fresh sawdust and sand, and 7 days ahead of that planted in F sand or medium sand.

3. Seed planted at a depth of 1 inch germinated much more rapidly and attained a higher final percentage of germination than did seed planted at depths of ½ inch or 2 inches.

4. Seed stored at 7° C. lost its viability sooner than did seed from storage that ranged either from 23°–32° or 7°–55° C.

BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING
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FRUIT SHAPE OF WATERMELON AS AFFECTED BY PLACEMENT OF POLLEN ON STIGMA

LOUIS K. MANN[1]

Introduction

Misshapen fruits in commercial watermelon plantings sometimes constitute a significant loss. Although the exact causes of asymmetry are not known, genetic factors, temperature or moisture gradients, mechanical injury, and other agents may be involved. In most plants the set and development of fruit is controlled somewhat by natural growth-regulating substances, whose activity and distribution are closely associated with pollination, growth of pollen tubes, and fertilization. The present study aimed to determine whether pollen-grain distribution on the stigmas significantly affects the fruit shape.

Since the three stigmas and carpels of the watermelon are radially arranged, pollen distribution among the three stigmas was studied with relation to radial symmetry. Pear- or top-shaped fruits were not studied.

MATERIAL AND METHODS.—Watermelons (*Citrullus vulgaris*) of the Blue Ribbon variety were grown for this experiment at Davis, California, in 1942.

Pistillate flowers were covered with cloth bags the day before anthesis. Pollination was carried out between 8:00 and 10:00 A.M. the next morning, since pollen was difficult to obtain later in the day. The flowers were then labeled and bagged, as described by PORTER (3).

The flowers were pollinated in three ways: (*a*) pollen applied to all three stigmas; (*b*) one stigma cut off near its base and pollen applied to the other two; (*c*) two stigmas removed and pollen applied to the one remaining.

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Pollen was spread abundantly over the stigmatic surfaces but was kept from the cuts where stigmas had been removed. In several melons, lanolin was spread on the cut surfaces to prevent pollen-grain germination. Since some injury resulted, however, the practice was not continued. Four melons so treated which matured (fig. 3, *C*, *K*; fig. 4, *B*, *P*) showed essentially the same seed distribution as if no lanolin had been used. The ovary was gently marked along one side with India ink to show excision of the stigmas, these marks later being renewed once.

Material for anatomical examination was fixed in Craf V (4). To trace pollen tubes, sections were stained with martius yellow-lacmoid blue (2). Other material was stained in iron haematoxylin.

Melons were harvested at or near maturity. The shape was recorded, especially near the blossom end, where deformity was most common. Shape and carpel size were also shown by a median cross-sectional tracing. The three carpels were then separated and their seeds counted. Empty seed coats were avoided, since they develop in parthenocarpic fruits and do not indicate fertilization (5).

The position of the carpels, which is not immediately obvious, must be ascertained for seed counts. In a cross section of a mature fruit, three conspicuous lines run from near the center almost to the edge, dividing the melon into three pie-shaped sectors, which, however, do not correspond to the three carpels. There is no obvious boundary between adjacent carpels. Figure 1*A* is the enlarged cross section of a fruit about 1.5 cm. in diameter. The broad ink marks indicate the limit of a single carpel. In figure 1*B* such a carpel has been cut free and teased apart to show the infolded edge of the car-

pel wall and placental region bearing the ovules. Each stigma is directly above its carpel.

Because of a late crop, some melons had to be harvested before maturity.

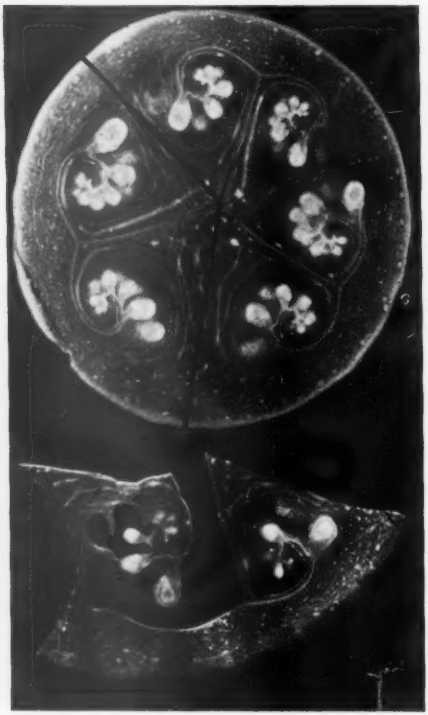

FIG. 1.—Top, cross section of young ovary, with limits of single carpel indicated by ink lines; bottom, single carpel cut free along lines indicated in top and teased apart to show infolded carpel, bearing the ovules.

Several were lost while small, being cracked and rotted because of extreme distortion. In figure 2*A* a single carpel (facing the observer) was unpollinated; in *B*, two carpels.

Investigation

GROWTH OF POLLEN TUBE.—Of primary importance was the direction of growth of the pollen tube. Does it necessarily fertilize an ovule located in the carpel on whose stigma the pollen grain was placed; and is the stimulative effect of pollen, pollen-tube growth, and fertilization confined mainly to the carpel where these processes occur?

The structure of the stigma, style, and ovary of the flower determines somewhat the path of the pollen tubes. The pistillate flowers have an inferior, usually tricarpellary, ovary, with a short thick style bearing three large stigmas.

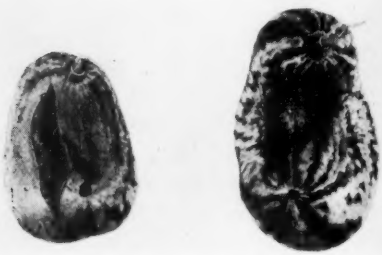

FIG. 2.—Immature melons showing cracking caused by unequal rates of growth among carpels: left, single carpel facing the observer was unpollinated; right, two carpels.

KIRKWOOD (1), studying the cucurbitaceous genera *Melothera*, *Micrampelis*, and *Cyclanthera*, described a stylar canal; usually the pollen tubes move over the surface of the stylar lobes and enter the ovary through the canal, although when tubes are numerous they may penetrate the surrounding tissues. Once within the ovary, the tubes grow along the surface of the carpel walls and placentae to the ovules.

Watermelon has no stylar canal; the pollen tubes penetrate a solid trilobed mass of tissue extending through the upper portion of the ovary. Once within the ovary, the tubes travel over the carpel walls and placentae, which are covered with a rather distinct surface layer of starch-filled cells, as described for related

genera by KIRKWOOD (1). These surface layers become fused in *Citrullus vulgaris* after fertilization. Thus, anatomically there is no obvious reason why pollen tubes growing from a single stigma should not reach ovules in all three carpels.

In serial sections of ovaries where only one stigma had received pollen, tubes could be traced into all three lobes of the conducting tissue. They then passed into the ovarian cavity, whence they might reach any of the placental regions. Their distribution to those regions in the three carpels is, however, not random. Most pollen tubes grow directly downward from the stigmas where pollen grains were placed; thus a grain is far more likely to bring about the fertilization of an ovule of the carpel where it was deposited than an ovule of any other carpel.

pollinated. A median cross-sectional slice of the melon is shown, the figures below each diagram indicating how many seeds were found in the carpels. In *A*, *E*, *F*, and *H* the last two figures indicating seed count are in one line separated by a comma. In *C* the seeds of only one carpel were counted. These melons varied greatly in shape. Most of the radial asymmetry occurred near the blossom end, one carpel being slightly flattened here and the blossom scar being located to one side of center. This was true of seven melons (*B*, *C*, *D*, *G*, *H*, *I*, *J*). The displacement of the blossom scar was measured as carefully as possible. For the seven it averaged 2.5 cm., with 3.0 cm. off center as maximum.

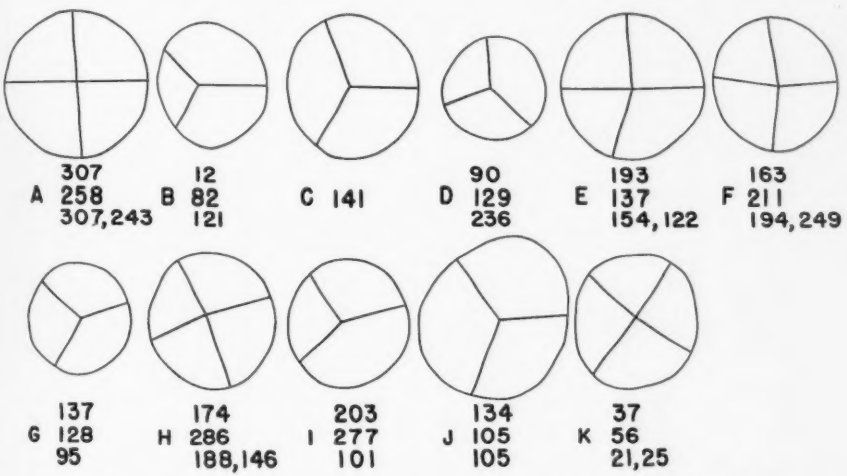

FIG. 3.—Median cross-sectional diagrams of control melons, showing area of carpels. Numbers below show seed count for each carpel. *A*, *E*, *F*, *H*, and *K* each have four carpels.

SHAPE OF FRUITS AND DISTRIBUTION OF SEED.—As already indicated, flowers were pollinated in three ways. Figure 3 shows the control group of melons where all stigmas (four in several cases) were

Judging from seed counts, the asymmetry may have resulted partly from differing amounts of fertilization in the carpels. In *B*, *D*, and *G* the smallest carpel (to the left in each diagram) contained the fewest seeds. In the other melons, the seed count does not refer to any specific carpel in the cross-sectional

diagram; and large differences in seed count are associated with differences in carpel size. Melons *A* and *K* are radially quite symmetrical, though *A* contains several times as many seeds as *K*. Melon *K*, however, was pear-shaped, all carpels to the left-hand carpel and the remaining numbers referring unspecifically to the other two.

All these melons except *F*, *H*, *M*, and *P* were asymmetrical to varying degrees. The blossom scar of the misshapen mel-

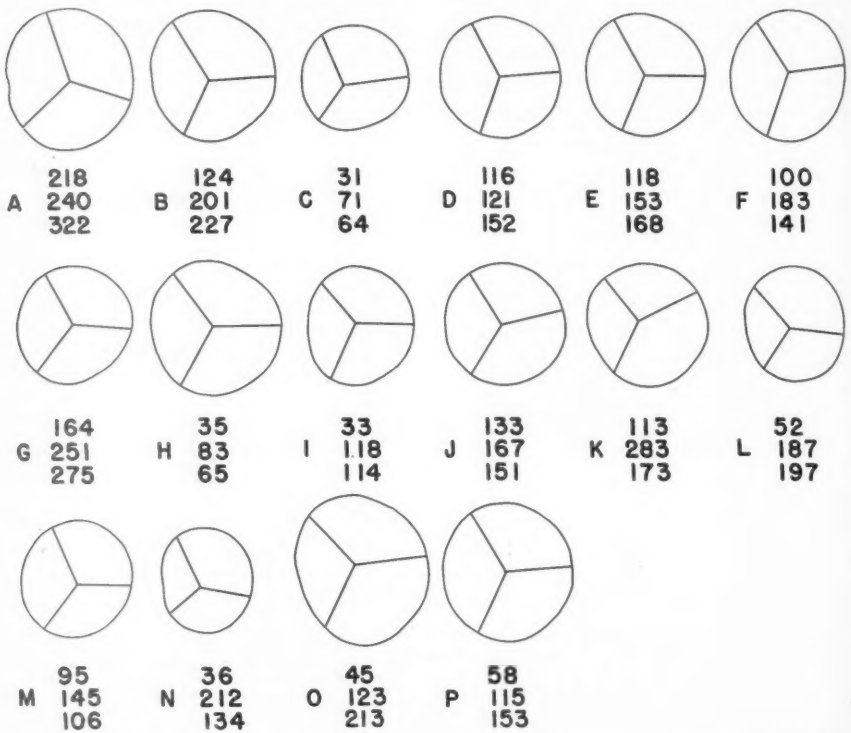

FIG. 4.—Median cross-sectional diagrams of melons pollinated on two stigmas only. Carpels at left were unpollinated. Upper number below each melon indicates seed count for unpollinated carpel; lower numbers refer unspecifically to the other two carpels.

being small toward the stem. Apparently shape is influenced more by relative number of developing seeds per carpel than by total number.

Figure 4 diagrams the melons in which one stigma was cut off and the remaining two were pollinated. The unpollinated carpel is always shown on the left. The numbers below indicate the number of seeds, the uppermost number referring

ons averaged 2.54 cm. off center, with 5 cm. as a maximum. Melon *N* was much curved, the blossom scar being displaced toward the stem end about 3 cm. from the apex. Except as just noted, most melons were misshapen, especially near the blossom end, where the unpollinated carpel was flat or sunken.

Figure 5 shows melons developed from flowers that had two stigmas removed

(three removed in *I*) and the remaining stigma pollinated. In each diagram the pollinated carpel is at the left; and of the numbers below, the uppermost indicates the seed count in the left-hand carpel, while the remaining numbers refer unspecifically to the other two. Whereas melons *G* and *K* were symmetrical, the others curved or bulged on the side of be estimated. The percentage of pollen tubes so crossing, with its standard error for the melons pollinated on two stigmas (fig. 4), is 20.78 ± 1.69 per cent; for the melons pollinated on one stigma (fig. 5, melon *I* excluded) it is 21.87 ± 1.40 per cent.

For better evaluation of asymmetry, the carpel areas of the melons in figures

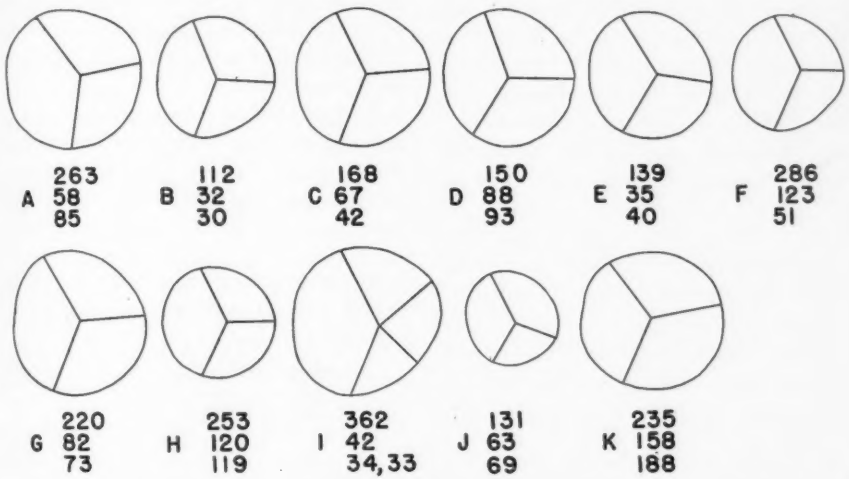

FIG. 5.—Median cross-sectional diagrams of melons pollinated on one stigma only. Pollinated carpel on left. Upper number below each melon indicates seed count for pollinated carpel; the other two numbers refer unspecifically to the remaining carpels.

the pollinated carpel; their blossom scars were pushed off center away from it, averaging 3.14 cm. off center, with a maximum of 5 cm. Melons *B* and *F* were so curved that the blossom scar was displaced 2.5 cm. and 5 cm., respectively, toward the stem end from the extreme apex. This group was more misshapen than the two groups previously discussed.

From the seed counts of pollinated and unpollinated carpels (figs. 4, 5) the percentage of pollen tubes that cross from a pollinated to an adjacent unpollinated carpel, bringing about fertilization, can 4 and 5 were measured with a polar planimeter. In the melons where two carpels were pollinated (fig. 4) the unpollinated carpels have a mean area (in arbitrary area units) of 69.41 units each; the pollinated, 83.51 units. In the melons where only one carpel was pollinated (fig. 5) the unpollinated carpels each have a mean area of 70.61 units; the pollinated, 122.27 units. Applying the *t*-test for unique samples, in both cases the difference in cross-sectional area between pollinated and unpollinated carpels is significant beyond the 1 per cent level.

Summary

1. The stigmas of the epigynous and usually tricarpellary flower of watermelon are distinct from one another. The conducting tissue of the style forms a solid three-lobed column, each lobe connecting a stigma with the carpel immediately beneath.

2. As shown by microscopic examination of this column, the pollen tubes growing downward from a specific stigma tend to be confined to the lobe of the conductive tissue, which is topped by the stigma where the pollen grains were placed. Certain pollen tubes turn laterally, however, both in the conductive tissue of the style and in the ovarian cavity, where they grow over the epidermal surface. Thus a pollen grain may bring about the fertilization of an ovule in a carpel other than the one on whose stigma it was placed.

3. According to seed counts from melons pollinated on only one or two stigmas, some 21–22 per cent of the pollen tubes will move laterally into each adjacent carpel.

4. The stimulus for enlarging a carpel is due, at least in part, to pollen tube growth and/or fertilization of ovules. This stimulus tends to be confined to the carpel where these processes take place. Although some pollen tubes cross from one carpel to another, large inequalities of pollen distribution among stigmas will cause differential growth among the three carpels, the fruit developing radial asymmetry, especially at the blossom end.

5. Analyzed by the *t*-test for unique samples, the mean difference in cross-sectional area between pollinated and unpollinated carpels of mature melons is significant beyond the 1 per cent level.

The writer is grateful to Dr. GLEN N. DAVIS for material and for helpful suggestions.

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MEGAGAMETOPHYTE OF CLINTONIA[1]

FRANK H. SMITH

Development of the megagametophyte of *Clintonia borealis* has been described by R. W. SMITH (3). According to his description, two unlike daughter nuclei are produced in the first reduction division. The nucleus formed at the chalazal end of the spindle consists of a dense chromatic mass, while that at the micropylar end develops into a typical interphase nucleus. As a result of the second division the chalazal nucleus produces two groups of daughter chromosomes which remain compacted into dense chromatic masses. The micropylar nucleus divides at the same time to form both a normal nucleus at the micropylar pole of the spindle and another mass of chromatic material at the opposite pole. Thus, of the four megaspore nuclei formed, the three inner ones are degenerate. No cell walls are formed following either division. The functional nucleus divides twice to form the nuclei of the egg apparatus and a single polar nucleus. One or more bodies representing the degenerate megaspore nuclei persist in the cytoplasm of the primary endosperm cell.

The reliability of these observations and interpretations has been questioned by many investigators, particularly by those interested in determining a system of classification of the different types of megagametophytes. SCHNARF (2) states: "A reinvestigation is needed, since the alleged course of development does not agree with that of closely related plants." MAHESHWARI (1) attempted to interpret the figures published by SMITH in terms of the *Fritillaria*-type megagametophyte. Much of the doubt cast upon SMITH's observations is probably due to the fact that he illustrated principally only telophase stages of most of the nuclear divisions involved.

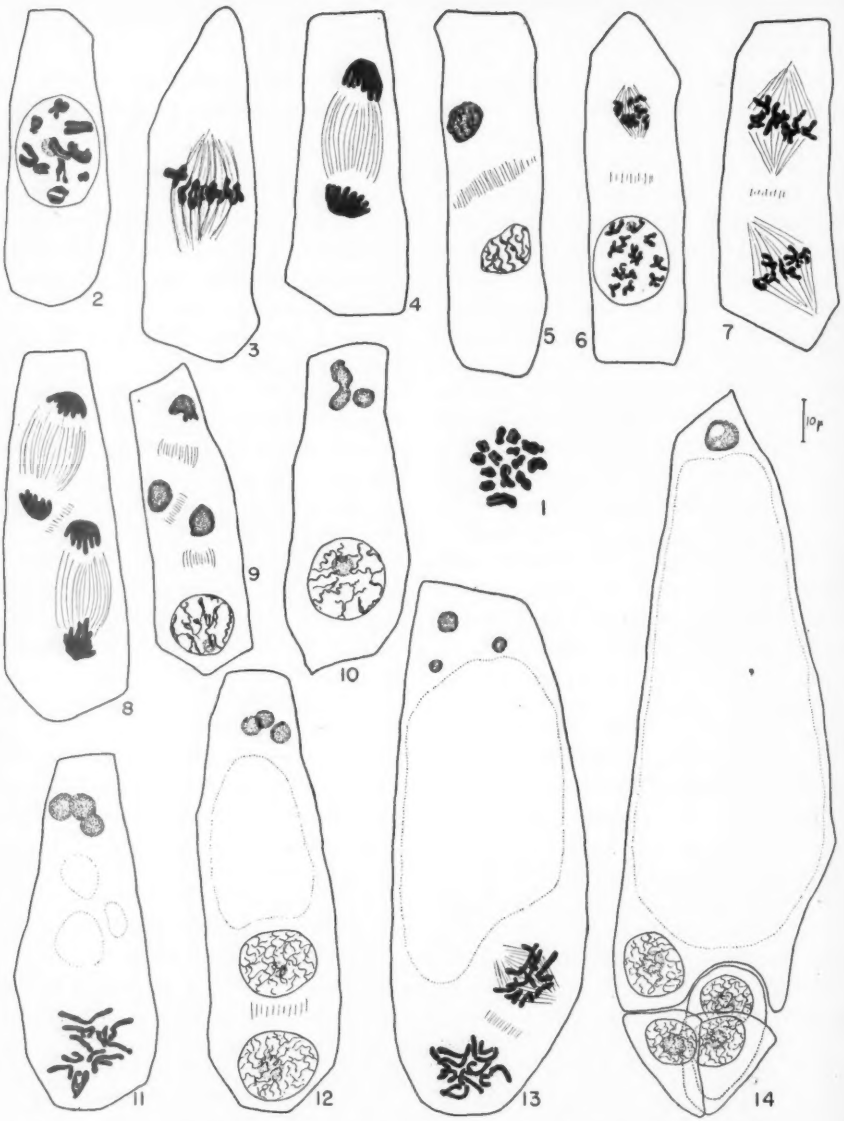
In view of the confusion in the literature regarding the megagametophyte of this genus, and the scarcity of critical stages observed by SMITH, material of *Clintonia uniflora* (Schult.) Kunth. was collected for study. The ovaries were fixed in Navashin's solution, dehydrated, and imbedded by the tertiary butyl-alcohol technique. Sections were cut 10-30 μ and stained with safranin and fast green.

Observations

The haploid number of chromosomes in *C. uniflora* is 14 (figs. 1, 11). The archesporial cell is hypodermal and functions directly as the megaspore mother cell (fig. 2). The chromosome pairs are arranged regularly at the equatorial plate of the first reduction division (fig. 3). This division proceeds in a normal manner up to the point where the chromosomes are compactly grouped at the poles of the spindle (fig. 4). Here, however, the two groups behave differently. The chromosomes at the pole of the spindle nearest the micropylar end of the mother cell undergo the usual telophase changes and become dispersed to form a typical interphase nucleus (fig. 5), while those at the chalazal pole remain compactly grouped because of suppression of the telophase. The midportion of the spindle persists in the cytoplasm, but no indications of a cell plate, as described by SMITH, were observed.

It seems likely that the telophase of the first division is merged with the prophase of the second and that both stages are rapidly completed. No prophase stages of the second division were de-

[1] Published with the approval of the Monographs Publication Committee, Oregon State College, as Research Paper no. 77, School of Science, Department of Botany.



FIGS. 1–14.—Fig. 1, meiotic metaphase I in microspore mother cell. Figs. 2–14, development of megagametophyte: fig. 2, diakinesis; fig. 3, metaphase I; fig. 4, late anaphase I; fig. 5, telophase I; fig. 6, prophase II; fig. 7, metaphase II; fig. 8, late anaphase II; fig. 9, late telophase II; fig. 10, megaspore containing one functional and three degenerate megaspore nuclei; fig. 11, metaphase of division of megaspore nucleus; fig. 12, late telophase of division of megaspore nucleus; fig. 13, metaphase of second division of megaspore nucleus; fig. 14, nearly mature megagametophyte.

scribed by SMITH, and very few were found in the present study. The compactly arranged chromosomes at the chalazal end of the cell separate from one another while the nucleus at the micropylar end passes rapidly through the usual prophase stages (fig. 6). The upper spindle develops before the lower one, but the metaphase is reached at approximately the same time in both (fig. 7). Thus, in spite of the differences in behavior of the micropylar and chalazal groups of chromosomes of the first reduction division, the spindles of the second division are both normal. They are usually arranged along approximately the same axis. So far as could be determined, each spindle contains the haploid complement of 14 chromosomes. The chromosomes then separate regularly into daughter halves, which move to opposite poles of each spindle (fig. 8). As in the first division, the telophase is suppressed in some of the chromosome groups. The chromosomes at the micropylar end of the outermost spindle undergo the normal telophase changes to form a typical metabolic nucleus (figs. 9, 10). The chromosomes of each of the three remaining groups become compactly arranged and appear gradually to fuse together and lose their identity in a mass of heavily staining material (fig. 9). Persistent fibers of both the first and second divisions are evident at this time but soon disappear without forming any cell plates.

The megaspores resulting from the reduction divisions are thus represented by one normal nucleus at the micropylar end of the cell and three masses of chromatic material at the center or the chalazal end. In *C. borealis*, SMITH noted that in every cell observed at this stage the three chromatic masses were present but that in later stages usually less than three were found. In *C. uniflora* frequent examples of the fusion of these bodies were observed (figs. 10, 11), although fusion does not always occur. Thus during later stages of the development of the megagametophyte there may be one, two, or three chromatic masses resulting from the fusion of all, some, or none of the degenerate megaspore nuclei. The appearance of one large and two smaller bodies in the same cell (fig. 13; and figs. 8, 9 of SMITH) is probably due to the fact that the products of the second reduction division in the chalazal end of the cell tend to become vacuolated and absorbed earlier than the other degenerate nucleus. During later stages any chromatic masses which are present become vacuolated and undoubtedly are eventually absorbed. They are still present, however, at the time of fertilization.

Division of the functional megaspore nucleus proceeds in the usual manner (fig. 11), and persistent spindle fibers of this division are evident between the daughter nuclei (fig. 12). These nuclei then divide (fig. 13), and cell plates are produced by the fibers of all spindles to cut out the egg apparatus and the primary endosperm cell. Thus the megagametophyte is composed of four cells (fig. 14), with the primary endosperm cell containing the polar nucleus and one, two, or three chromatic bodies representing the degenerate megaspore nuclei.

Discussion

Much of the confusion regarding the nature of the megagametophyte of *Clintonia* may possibly be traced to its unfortunate comparison by SMITH with the megagametophyte of *Oenothera*. SCHNARF (**2**) apparently misinterpreted this comparison and stated that "according to SMITH .... *Clintonia borealis* follows the *Oenothera*-type." SMITH emphasized

that, while the normal nucleus and the three degenerate nuclei produced by the reduction divisions represent four megaspores, there are no walls between them. The principal point of comparison of the two types seems to be that it is the micropylar megaspore nucleus in each case that functions to produce the quartet. The same, however, may be said of the *Gunnera-*, *Pyrethrum-*, *Majanthemum-*, and *Fritillaria*-type megagametophytes in SCHNARF's system of classification. The presence of the degenerate megaspore nuclei in the antipodal end of the megagametophyte indicates a closer relationship of *Clintonia* to these rather than to *Oenothera*.

MAHESHWARI (1) states, regarding SMITH's observations on *C. borealis*, "This satisfies the requirements of the *Oenothera*-type, and if SMITH's observations are correct *Clintonia* should be regarded as an interesting example of a monosporic tetranucleate embryo sac." The present work on *C. uniflora* shows that SMITH's observations are probably correct. He stated definitely, regarding the products of the reduction divisions, "No one doubts that these are megaspores, simply because three of them or their products disintegrate." This statement and his subsequent discussion indicate that he considered the megagametophyte to be tetrasporic, with three of the spores represented only by degenerate nuclei.

However, MAHESHWARI's interpretation of SMITH's illustrations in terms of the *Fritillaria*-type megagametophyte is not entirely incorrect. According to this interpretation the development follows the *Fritillaria*-type up to the completion of the secondary 4-nucleate stage. The two micropylar nuclei then divide to form the quartet, while the two chalazal nuclei fail to divide. Thus *Clintonia*, on the basis of this interpretation, would have a reduced megagametophyte of the *Fritillaria*-type with six nuclei instead of the normal eight. The present work has shown that a secondary 4-nucleate stage, in terms of the *Fritillaria*-type, does not exist in *Clintonia*.

It would appear from the observations of SMITH on *C. borealis*, and from the present investigation of *C. uniflora*, that *Clintonia* does have a megagametophyte reduced from the *Fritillaria*-type. However, the reduction is much greater than that postulated by MAHESHWARI. The latter discusses a reduction series in this type of megagametophyte that leads logically to the situation in *Clintonia*. He states that in *Gagea* the lowest nucleus of the secondary 4-nucleate stage does not divide, so that a 7-nucleate megagametophyte is formed, while in *Gagea ova* the chalazal pair of nuclei of the secondary 4-nucleate stage sometimes undergo no further division and the megagametophyte is 6-nucleate. In a footnote he adds that in *Caulophyllum robustum*, as described by MAURITZON, the three chalazal nuclei of the primary 4-nucleate stage fuse to form a triploid nucleus which does not undergo further division. The megagametophyte is thus 5-nucleate.

Clintonia shows the same situation as that described for *Caulophyllum*, except that in *Clintonia* the three chalazal megaspore nuclei are degenerate and do not always fuse.

Summary

1. Observations by R. W. SMITH on the megagametophyte of *Clintonia borealis*, which have been much doubted, are probably essentially correct, since *C. uniflora* follows the same pattern of development as he described for *C. borealis*.

2. *Clintonia* has a tetrasporic megagametophyte which probably represents a much reduced form of the *Fritillaria*-type. The nucleus of the megaspore mother cell divides to form four megaspore nuclei, of which the three toward the chalazal end are represented only by densely chromatic masses. All or some of these masses may fuse and then persist as one or more bodies in the cytoplasm of the primary endosperm cell. The micropylar megaspore nucleus divides twice, to produce the quartet of two synergids, the egg, and a single polar nucleus.

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HISTOLOGICAL AND CYTOLOGICAL RESPONSES OF ROOTS TO GROWTH-REGULATING SUBSTANCES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 557

WILLIAM M. CARLTON

Introduction

Gross responses of plant organs to growth-regulating substances have been studied widely during the past decade. Histological and cytological studies of the effects of such substances on stems and other aerial organs have been reported by many investigators (**1, 2, 4, 9, 11, 12, 13, 14, 15**). Studies of the effects of such substances on plant roots, however, have been limited largely to gross observations on the stimulation or inhibition of root elongation, the development of laterals, and the physiological activity of the roots. Only recently have the histological and cytological changes induced been considered.

According to THIMANN (**20**), NIELSON first demonstrated (in 1930) the inhibition of root elongation by the external application of auxins, using in his treatment a crude extract from *Rhizopus* cultures. Later (in 1934) KÖGL, HAAGEN-SMIT, and ERXLEBEN found that pure auxin a, auxin b, and indole(3)acetic acid had a similar effect. THIMANN found that indene(3)acetic acid applied externally also inhibited elongation, and he advanced the suggestion that perhaps this inhibition is a general property of growth-regulating substances.

ZIMMERMAN and HITCHCOCK (**21**) found that the normally unbranching aerial roots of grape could be induced to form numerous lateral roots and that elongation was retarded by treatment of the region with any of several chemicals in lanolin paste or by immersing the root tips in aqueous solutions of the substances.

LANE (**16**) found that indole(3)acetic acid is a specific inhibitor of root growth in young *Avena* seedlings and that the inhibition is accompanied by some thickening of the root. Stunting effects on roots also were reported by THIMANN, MARMER (**19**), MACHT and GRUMBEIN (**18**), and BONNER and KOEPFLI (**5**). These effects were studied chiefly on roots of cereal grains and legumes. BORGSTROM (**6**) investigated the effects of a number of growth substances on bulb-forming plants (*Allium cepa, A. fistulosum*, and *Crocus*), determining effective concentrations, toxicity limits, and gross morphological effects. He found that elongation of roots was inhibited by treatment with all but the most dilute concentrations, and inhibited elongation often was accompanied by enlargement of the root tips.

The effectiveness of growth-regulating substances in the induction of lateral root formation has been observed in various plants (**21, 20, 16, 19, 8, 6**). MARMER (**19**) reported excessive root-hair formation following treatment of wheat seedlings with indoleacetic, indolebutyric, and indolepropionic acids at pH 4.6 but not at pH 7.5.

The histological and cytological responses of roots of species of *Allium* to several chemicals has been investigated by LEVAN (**17**). BURSTRÖM (**7**) has discussed the morphological changes induced in roots of wheat following treatment with heteroauxin.

The present investigation was undertaken for the purpose of extending information on the histological and cytological changes which occur in roots following treatment with various growth-regulating substances.

Material and methods

The roots used were those of bulb-forming monocotyledons. The species selected were *Allium cepa, Narcissus* (var. Paper White), and *Tulipa* (vars. John Ruskin and Louis XIV), but not all were treated with all of the chemicals used.

Six growth-regulating substances were used: α-naphthaleneacetic acid, indole(3)acetic acid, indole(3)butyric acid, β-naphthoxyacetic acid, α-naphthyl acetamide, and tryptophane. They were used in concentrations of 0.001 or 0.002%, 10 or 20 p.p.m., dissolved in a three-salt nutrient solution. Because of the difficulty in getting some of the substances into solution in an aqueous medium, all but tryptophane and naphthyl acetamide were first dissolved in small amounts of ethyl alcohol, in a manner similar to that employed by MACHT and GRUMBEIN (18); later, measured amounts of the alcoholic solutions were pipetted (with constant agitation) into definite volumes of nutrient solution to give the desired concentrations. The naphthyl acetamide, being relatively insoluble in ethyl alcohol, was put into solution in hot n-butyl alcohol. Tryptophane was dissolved directly in the measured volume of nutrient solution.

The bulbs were rooted by suspending them on wire-gauze racks over containers of nutrient solution so that the basal portions were submerged. When the roots had reached a satisfactory length, 1 inch or more in case of *Allium* and *Narcissus* and somewhat less in the case of *Tulipa*, individual bulbs were removed from the racks, rinsed with water, and suspended in tumblers so that the roots were completely immersed in nutrient solution to which the respective substances had been added.

Length of treatment varied from 24 to 72 hours, being generally 48 hours. At the conclusion of the treatment the bulbs were removed from the growth-substance solution, rinsed in distilled water, and transferred to tumblers of regular nutrient solution. Roots were cut at even intervals from 24 to 216 hours after the bulbs were replaced in nutrient solution, and fixed in Navashin's solution under reduced pressure.

The fixed roots were dehydrated by the tertiary butyl-alcohol method, imbedded in paraffin, sectioned, and stained in a modification of Flemming's triple stain. All sections were cut at 15 μ, with the exception of the longitudinal sections of the *Tulipa* roots. These, because of their small size, were sectioned at 10 μ.

Observations

GROSS RESPONSES

No definite responses of the roots were noticed for approximately 24 hours after replacing the bulbs in nutrient solution following treatment with the growth substance solutions; but between 24 and 72 hours after their return, several changes were noted.

All the chemicals in the concentrations used resulted in inhibition of root elongation, either temporary or permanent. Following treatment with indoleacetic acid the roots soon began to elongate, and in some cases the root tips grew as much as 2 inches during the subsequent 3 weeks. The roots treated with the other chemicals showed no later elongation.

Often a swelling was observed near the root tip; the time of occurrence and the amount varied in the different species. *Allium* showed the quickest response, but *Narcissus* reacted almost as rapidly. The amount of terminal enlargement in

the roots of *Tulipa* was so slight as to be hardly discernible, and histological examination of longitudinal sections were required to determine it.

The terminal swellings began about 24 hours after replacement of the bulbs in nutrient solution. Their size increased and reached a maximum in 1–3 days.

The enlargements which developed on roots of *Allium* and *Narcissus* as a result of treatment with the indoleacetic, indolebutyric, naphthaleneacetic, and naphthoxyacetic acids were similar in appearance and somewhat larger in diameter than were those induced by tryptophane. They were frequently twice the diameter of the root and sometimes scarcely longer than thick, while those induced by tryptophane were slender and often $\frac{1}{2}$ inch or more in length.

About 200 hours after treatment, the enlargements at the tips of *Narcissus* roots treated with naphthaleneacetic began to produce root hairs. At 264 hours the enlargements were almost completely covered with them, none more than a small fraction of a millimeter in length. Root hairs were not induced by the other chemicals, nor were they formed on roots of the other species.

Root primordia and secondary roots developed only on *Allium*. This genus normally forms secondary roots, but there were more primordia, and these were closer to the terminal meristem than in untreated bulbs. The secondary roots were much stunted and enlarged, and some of them had primordia (fig. 4).

Histological and cytological responses

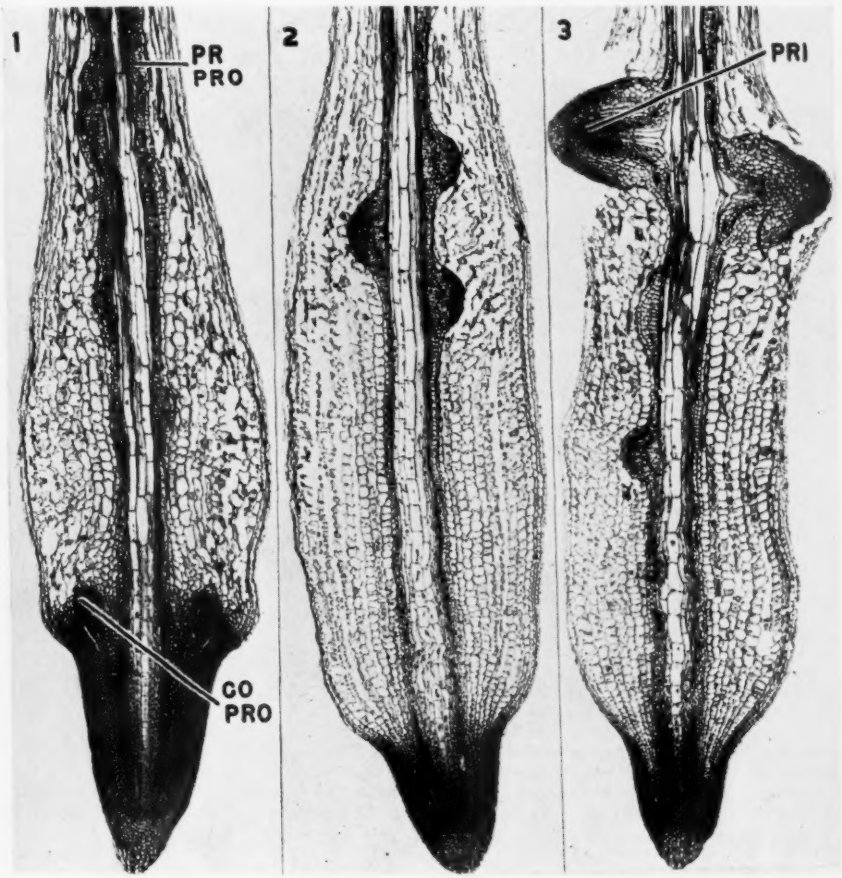
In *Allium* the characteristic enlargement of the root tip following treatment reaches maximum size within 48 hours. Transition from normal tip to swollen area is rather gradual and covers about 20 cell lengths. Within 48 hours the pericyclic cells for some distance back from the apex of the root undergo transverse divisions which result in the formation of longitudinal rows of short, meristematic cells, which divide in several planes (figs. 1, 2). Tangential division of the cells of the pericycle becomes prominent in 72 hours, and within 96 hours definite root primordia are developed. About 264 hours are required for the primordia to grow through to the epidermis (fig. 8). Within 360 hours many have begun to emerge (figs. 3, 5, 7). Some are much enlarged and in turn have formed primordia (fig. 4).

While cell proliferation is much more frequent in the pericycle than in any other tissue of the root, it may also occur in other tissues, such as the cortex (figs. 1, 6). Proliferation, when it occurred, was in the form of a band centrifugal to the stele and extending almost to the epidermis (figs. 1, 6). Such divisions in the cortex of the embryonic tip occur early in ontogeny. They are at first radial or diagonal, but in older material many divide tangentially. Division is not pronounced in the larger cells of the swollen region. Some division also occurs in the mature cells far above the terminal enlargement.

Roots of John Ruskin and Louis XIV *Tulipa* do not develop noticeable swollen tips after treatment. The earliest response is the altered cell polarity in the immature cortex. As early as 48 hours after treatment, and in all cases thereafter, these cells show evidence of recently completed divisions in all planes. Close to the tip the divisions are predominantly radial or diagonal, but at higher levels many of them are tangential (figs. 9, 10). For the first 48 hours after treatment the divisions are limited almost exclusively to the meristematic re-

gion, but after 72 hours divisions in several planes occur in mature cells (fig. 10).

formed remaining slender and undergoing no enlargement. Subsequently the pericyclic cells divide further and form



FIGS. 1–3.—Longisections of *Allium* root tips treated with 0.001% naphthaleneacetic acid: Fig. 1, 120 hours after treatment, showing proliferation of pericycle and cortex. Fig. 2, 216 hours, showing root primordia. Fig. 3, 360 hours, showing primordia emerging. Figs. 2 and 3 show transition from normal to enlarged regions. *Pr pro*, proliferation of pericycle; *Co pro*, proliferation of cortex; *Pri*, root primordium.

Transverse divisions are initiated in the pericycle approximately 72 hours after treatment and continue for 72 hours or more. These divisions are confined to limited areas. Tangential divisions first occur about 168 hours after treatment, the daughter cells thus

ridges of densely packed cells which often extend 200 μ or more along the stele. Whether root primordia are initiated is uncertain. No definite organization was seen in the ridges of meristematic cells, but it is possible that root primordia might eventually differentiate. Iso-

lated cells of the endodermis undergo tangential division within 96 hours after treatment, and within 144 hours many have divided.

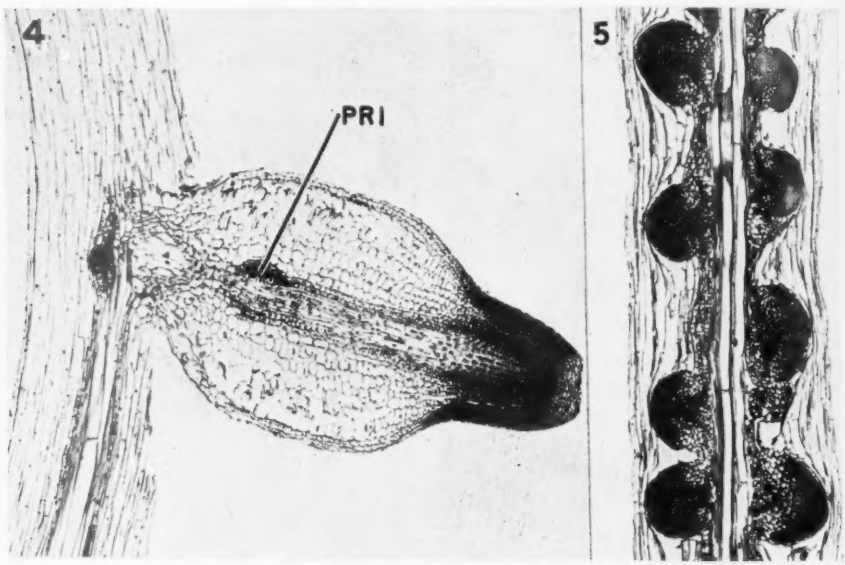
In *Narcissus* roots no terminal swelling appears until 72 hours after treatment, and the maximum size is not reached for approximately 144 hours. Few or numerous root hairs develop on the tumor after 200 hours. Their maximum length never exceeds a small fraction of a millimeter.

Some division occurs in mature cells of the cortex, particularly in the early stages. No response was observed in either the pericycle or the endodermis.

INDOLE(3)ACETIC ACID.—Treatment with 0.001% for 24 hours was applied to John Ruskin *Tulipa* and *Narcissus* in the manner previously described. Roots were collected and fixed at the conclusion of the treatment, and at regular intervals up to 192 hours after the bulbs were replaced in nutrient solution.

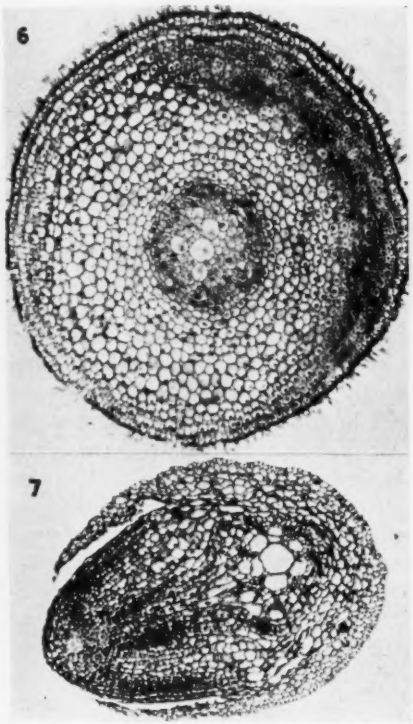
Treatment does not result in tumor formation on roots of the John Ruskin variety. In fact, little variation from normal can be detected. Divisions in the cortex of the meristematic region appear about 12 hours after treatment and eventually increase in number. These divisions at first are mostly radial, but later numerous tangential and diagonal ones occur. The daughter cells do not enlarge, and division in any cortical cell is completely independent of division in the adjacent cells.

Radial divisions in both pericycle and endodermis begin as early as 12 hours after treatment. Cells of the endodermis show tangential divisions 48 hours after treatment, and about 24 hours later



FIGS. 4, 5.—Longisections of mature regions of *Allium* roots 360 hours after treatment with naphthaleneacetic acid. Fig. 4, induced lateral root with young primordium developing. Fig. 5, section about 3 cm. above root apex with numerous root primordia.

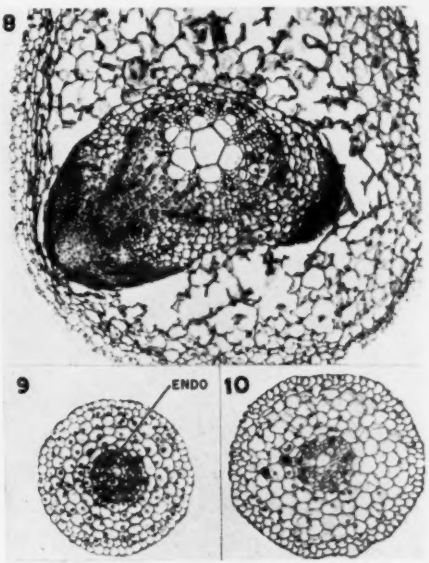
those of the pericycle also. At the same time, or perhaps somewhat earlier, the pericyclic cells undergo transverse division, resulting in rows of cubical cells



FIGS. 6, 7.—Transection of *Allium* root treated with naphthaleneacetic acid: Fig. 6, 120 hours after treatment, cut through base of tumor to show proliferation of cortex (cf. fig. 1) and early divisions in pericycle and endodermis. Fig. 7, 360 hours after treatment, showing emerging root primordium and development of vascular connections.

which are densely filled with cytoplasm and apparently meristematic. There are continued radial and tangential divisions in these tissues 120–192 hours after treatment, and the cortical cells adjacent to the endodermis also are affected. Proliferation is general, resulting in a compact band of four to six rows of cells around the stele. In more mature portions general proliferation does not occur, but localized regions of meristematic cells may indicate differentiation of root primordia.

Roots of *Narcissus* show no terminal enlargement at the conclusion of the

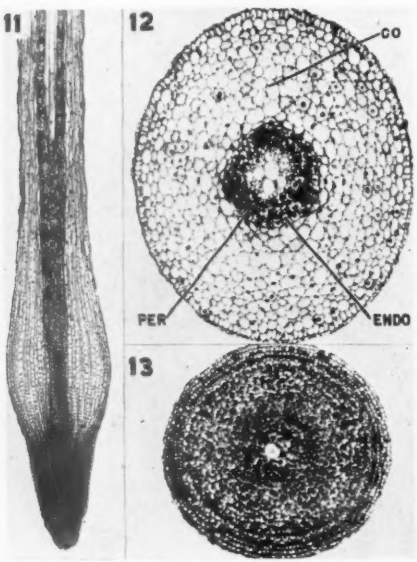


FIGS. 8–10.—Transection of roots treated with naphthaleneacetic acid: Fig. 8, *Allium*, 264 hours after treatment, showing developing primordia and torn cortex. Fig. 9, *Tulipa* Louis XIV, 120 hours, showing newly divided cells in endodermis and cortex and "apparent" binucleate condition in some cortical cells. Fig. 10, Louis XIV, 144 hours, showing effects of division in cortex and mitosis in mature cell. *Endo*, endodermis.

treatment, but swelling is initiated within 24 hours and reaches its maximum in about 48 hours. Tumor formation results from enlargement of the cells of the cortex, with no indication of increase in the number of rows of cells in a longitudinal section. The treatment exerts a temporary stunting effect, stopping elongation for approximately 96 hours; but this is of short duration and then elongation is resumed. With renewal of elongation, the

tumor is left farther and farther behind the growing tip. Apparently, however, no further change occurs in its cells, for they remain almost isodiametric.

A few tangential and radial divisions occur in the cortical cells. The peripheral four or five rows undergo less enlargement than those nearer the center, and as a result the swollen region is bounded by a band of compact cells which remain active longer than most of the others.



FIGS. 11–13.—*Allium* root: Fig. 11, 72 hours after treatment with indolebutyric acid, showing proliferation of pericycle and transition from normal tip to tumor. Fig. 12, transection through tumor, 72 hours after treatment with indolebutyric. Fig. 13, transection just below tumor, 168 hours after treatment, showing divisions of cortical cells in all planes. *Co*, cortex; *Endo*, endodermis; *Per*, pericycle.

Intercellular spaces, which occur even in the meristematic region of the root tip, are much larger in the swollen portion of the root. Mature cells are stimulated to divide in many instances, such divisions occurring as far as 16 mm. above the root tip, a considerably greater distance than is usual. In all instances division in mature cells occurs transversely.

INDOLE(3)BUTYRIC ACID.—Rooted bulbs of *Allium*, Louis XIV *Tulipa*, and *Narcissus* were treated with a 0.001% solution of this acid for 24 hours. Following treatment, roots were collected and fixed at 48-hour intervals up to and including 216 hours.

Swelling induced in *Allium* roots by this treatment reaches maximum size in approximately 24 hours, forming a spindle-shaped enlargement just above the root tip (fig. 11). First the pericyclic cells undergo repeated transverse divisions to form blocks of short meristematic cells. Tangential divisions follow shortly, forming a compact band several cells in thickness around the stele (fig. 12). This is accompanied by tangential divisions in the endodermis and, to some extent, by similar divisions of the inner cells of the cortex. The band thus formed varies in thickness from two to twelve cells and has an irregular external boundary. Development of this compact band of tissue is generally limited to the elongation and maturation regions. In the mature regions, within 72–216 hours after treatment, distinct areas of proliferation are formed. At first these are no more than compact groups of actively dividing cells, but later there are definite organization of tissues and differentiation of root primordia.

Mature cells of the cortex divide, but all divisions appear to be oriented normally in a transverse plane. Divisions in all planes occur in the immature cortical cells, the number progressively increasing. At 168 hours almost all the cells in the cortex of the meristematic region have undergone one or more divisions (fig. 13).

The only responses of roots of Louis

XIV *Tulipa* to treatment consist of a few radial and tangential divisions in the young cortical cells and a few transverse divisions in the mature region.

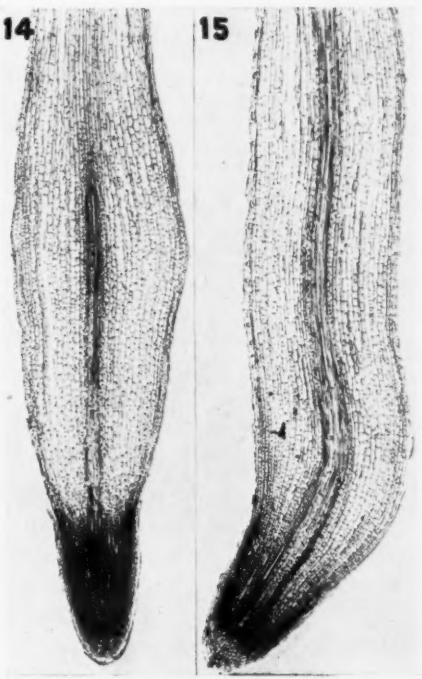
Roots of *Narcissus* show no pronounced swellings 24 hours after treatment, but these are obvious within 72 hours. They are formed by the enlargement of individual cells rather than by increase in their number. There is a temporary stunting of root growth which lasts for approximately 168 hours, after which there is some slight renewal of elongation. Both radial and tangential divisions occur in the cells of the immature cortex but never abundantly. There are also a few transverse divisions in the mature cortical cells.

Transverse sections through the tumor show that the five or six peripheral rows of cortical cells are much more compactly arranged than are those nearer the stele. Intercellular spaces become numerous and large. Development of a limited number of stubby root hairs occurs on the lower portion of the tumor 120–216 hours after treatment.

β-NAPHTHOXYACETIC ACID.—Bulbs of both varieties of *Tulipa* and *Narcissus*, with abundant root development, were treated for 48 hours with a 0.002% solution of this acid. Roots were collected and fixed at 24-hour intervals from 48 to 144 hours.

Narcissus roots usually respond to treatment by tumor formation (figs. 14, 15), but there are exceptions where no swelling occurs. The roots are apparently permanently stunted. The tumors are formed by the enlargement of cortical cells, chiefly in a radial plane. Transition from the normal root tip may occur gradually or abruptly, involving only a few cell lengths. Roots collected and fixed at 72 and 120 hours after treatment have sparse development of root hairs on the lower part of the tumor, and their maximum length is about 0.1 mm.

Transverse sections indicate that cell development and mitosis are normal during early phases (up to 96 hours), but in material fixed 120 hours or more after

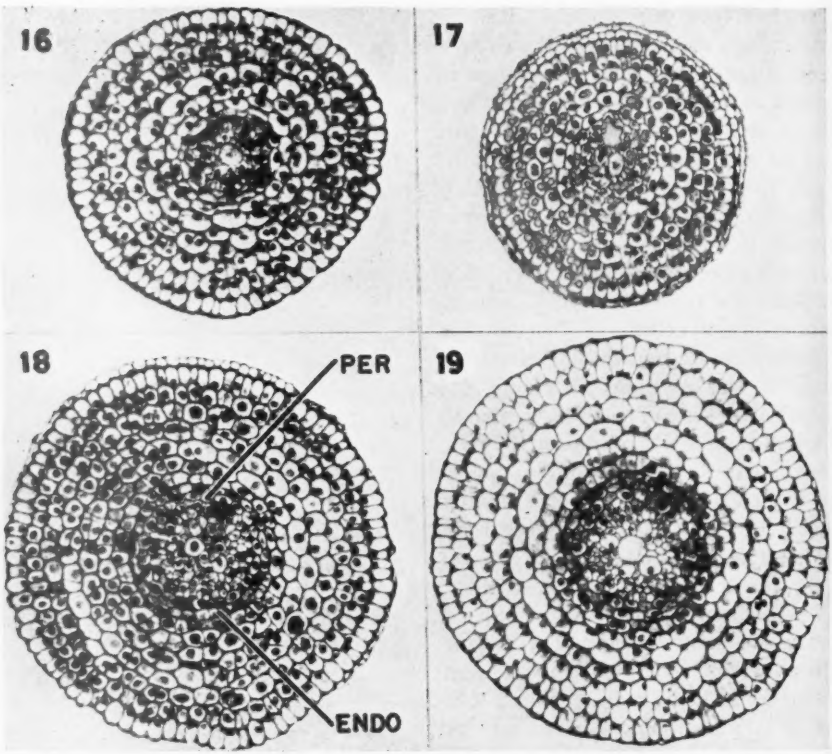


FIGS. 14, 15.—*Narcissus* root: Fig. 14, longitudinal section, 120 hours after treatment with naphthoxyacetic acid. Fig. 15, 144 hours after treatment with naphthyl acetamide.

treatment there are numerous divisions in all planes in the meristematic region. Mature cortical cells as far as 10 mm. from the root tip are stimulated to divide. Intercellular spaces occur in the swollen portion of the root. No changes were induced in the cells of the pericycle or in the stele of any root given this treatment.

Pronounced terminal swelling does not occur in roots of Louis XIV. Cortical

cells just back of the root tip undergo numerous divisions, but the daughter cells show little enlargement. The earliest divisions appear to be radial and occur mainly toward the periphery (figs. 16, 17), but of those occurring later, many are tangential (figs. 18, 19). Pericyclic cells are stimulated to divide transversely in certain regions, forming short rows of nearly isodiametric cells with large nuclei and dense cytoplasm. In older roots, killed 120 hours or more after treatment, both pericyclic and endodermal cells divide both radially and tangentially; the daughter cells thus formed remain densely filled with cytoplasm. These regions often appear to be associated with the protoxylem points, which might indicate early stages in the formation of root primordia; but development is so slight that no definite conclusions can be drawn.



FIGS. 16–19.—Transections of *Tulipa* Louis XIV roots treated with naphthoxyacetic acid: Fig. 16, 48 hours after treatment, showing numerous recently completed divisions in cortex. Fig. 17, 96 hours; similar divisions. Fig. 18, 120 hours; divisions in all directions in cortex and initiation of divisions in endodermis. Fig. 19, 144 hours; section cut at higher level, showing divisions in endodermis and pericycle. *Endo*, endodermis; *Per*, pericycle.

Mature cells of the cortex are stimulated to divide as far as 10 mm. from the root tip and in a number of planes.

Roots of John Ruskin *Tulipa* respond

similarly but somewhat more slowly than those of Louis XIV. Pericyclic cells divide frequently, and the daughter cells are filled with dense cytoplasm; but despite their meristematic appearance, no proliferation appears to take place.

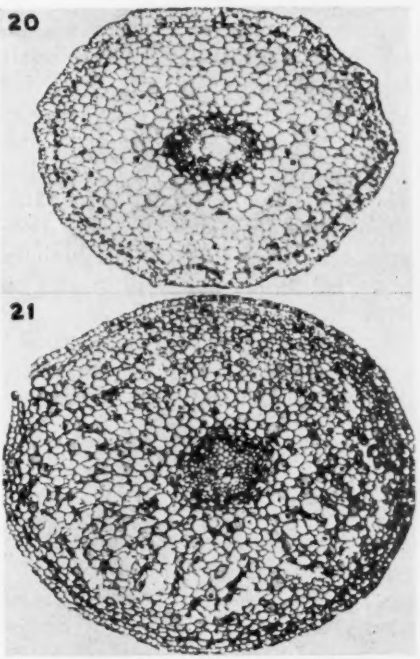
α-NAPHTHYL ACETAMIDE.—Rooted bulbs of *Allium*, Louis XIV *Tulipa*, and *Narcissus* were treated for 48 hours with a 0.001% solution. Roots were collected and killed at 48-hour intervals up to 192 hours.

Allium roots show considerable swelling, forming bulbous tumors. Lateral root primordia develop rather quickly, and mitosis occurs in cells throughout the terminal 10 mm. of the roots, both in the stele and in the cortex. The tumors formed on the roots are similar to those previously described and result from cell enlargement rather than from increase in cell number. Transition from the normal root tip takes place within 10–12 cell lengths. Few of the enlarged cells of the cortex show either recently completed divisions or mitotic figures, indicating little meristematic activity.

Root primordia are initiated within 48 hours following treatment, but the number at this time is few; at 144 hours after treatment their number has increased considerably. The position of the primordia appears to have no effect on their size or rate of development, since those nearest the root tip are not necessarily the youngest or the smallest. Within 192 hours after treatment, some primordia have pushed halfway through the cortex. The steps in the development of primordia are essentially the same as those previously discussed, but none developed sufficiently to emerge from the main root.

Some enlargement of the hypodermal cells (fig. 20) occurred in *Allium*. The enlargement, however, is general and not limited to regions over the protoxylem points, as was described by BURSTRÖM (7) for wheat.

Louis XIV *Tulipa* shows relatively little response to treatment. A very slight swelling of the root tip occurs,



FIGS. 20, 21.—Fig. 20, *Allium* root, 48 hours after treatment with naphthyl acetamide, showing some enlargement of hypodermal cells. Fig. 21, *Narcissus*, 96 hours, showing disruption of cortex and compact layer of cells around periphery.

caused by limited enlargement of the cortical cells, which in the meristematic region show numerous divisions, at first radial and later both tangential and diagonal. The resulting daughter cells do not enlarge or show further development.

Cells of the pericycle undergo transverse division, and the daughter cells contain dense cytoplasm and have large nuclei, giving them the appearance of meristematic cells; but no proliferation

occurs and no root primordia are initiated. Mature cortical cells are stimulated to divide, and a number were observed in various stages of mitosis.

Within 48 hours after treatment, roots of *Narcissus* show prominent swellings a short distance above the tips, although pronounced enlargement does not always occur (fig. 15). The tumors result from cell enlargement only, involving chiefly the inner cortical cells—the peripheral cells remaining normal size. Continued enlargement of the cortical cells results in the formation of large schizogenous air spaces (fig. 21). Pericyclic cells show none of the activity shown by *Allium* and *Tulipa*, and no root primordia are initiated.

TRYPTOPHANE.—Roots were exposed to a 0.002% solution for 48 hours, after which the bulbs were returned to a balanced nutrient solution. Roots were killed at regular intervals from 24 to 192 hours after treatment.

Roots of *Allium* show little morphological change after treatment with tryptophane. No terminal swelling occurs, and there is only slight effect on cell polarity, since almost all mitotic figures are oriented normally. Up to 48 hours few of the young cortical cells show signs of recently completed division, although such divisions become more frequent later.

The endodermal cells divide tangentially, beginning about 72 hours after treatment, but become numerous only after 96 hours. The pericyclic cells, however, do not undergo the usual transverse divisions reported for the preceding substances. Pericyclic and endodermal cells gradually become less densely filled with cytoplasm as they mature. Neither initiation of root primordia nor proliferation of the pericycle takes place.

Mitosis occurs only in the meristematic region, the terminal 1.5–2 mm. of the root. In none of the material observed were the mature cells of the cortex stimulated to divide. Maturation of the tissues, particularly those of the stele, seems to occur more slowly in roots treated with tryptophane than in control material.

Roots of both tulips respond to tryptophane much as do those of *Allium*. Perhaps division in the endodermal and cortical cells occurs somewhat earlier in the John Ruskin. Approximately 72 hours after treatment, however, certain regions of the pericycle undergo transverse cell division, forming rows of short, almost isodiametric cells with dense cytoplasm. Tangential divisions take place in these cells but never become abundant. These responses occur at some distance proximal to the root tip.

The pericycle continues development from 96 to 192 hours after treatment, but the development is limited to definite regions. At first there is a mass of meristematic cells which shows no definite organization, but later there is a certain amount of organization. In a few instances the development of the proliferation is inward, resulting in some displacement of the vascular elements. Some cells of the proliferation take on the structure and appearance of wound tracheids, and it is possible that this leads to the formation of vascular tissue within the proliferation and the formation of vascular connections with the xylem of the root.

Narcissus roots develop slight spindle-shaped swellings near the tips, increasing the root diameter by about one-half. Cell polarity in the meristematic region is unaffected by treatment, and practically all the divisions are transverse. Pericyclic cells lose their dense cytoplasm in the elongation and maturation regions, and few radial and tangential divisions

occur. Mitosis is not promoted in the cells of mature tissues.

Discussion

The responses of these roots to treatment with the various growth-regulating substances extend, and to some extent agree with, the histological observations of LEVAN (17) and the general observations of BORGSTROM (6), GAVAUDAN (10), LANE (16), and others. Formation of terminal tumors on treated roots confirms the discussion and the illustrations presented by BORGSTROM. However, some of the data are not in agreement with the results of LEVAN and BURSTRÖM. The latter states that the rate of mitosis (in roots of wheat) is unchanged by the addition of heteroauxin (indole(3) acetic acid), but that such treatment does exert an influence on the direction of cell divisions, some being changed from transverse to radial or tangential; and thus an increase in the number of rows of cells is brought about, giving an increased root diameter. LEVAN states that "it will be seen from the longitudinal section that a larger number of cell rows is present than normally. This is due to the fact that the meristematic cortical cells under the influence of the growth substances start numerous mitoses. These mitoses are oriented in the transverse axis of the root, tangentially or radially." He then states that such divisions are not a necessary condition for the subsequent swelling of the cells, and that frequently the divisions are lacking.

In the present investigation there was no indication of increase in the number of vertical rows of cortical cells in the transition from the normal to the enlarged regions of the roots (figs. 2, 3, 11, 14, and 15), and few cases of cell division in any plane were observed in the cortical cells of the tumors. Divisions in many planes frequently were seen in the immature cortex below the tumor, however, and mitosis in mature cortical cells above the tumor was not uncommon. It is evident that treatment does affect cell polarity, for irregular divisions in the root tips did not occur in control material.

BURSTRÖM also described a hypertrophy of the hypodermal cells over the protoxylem strands in the mature region of roots of wheat seedlings, the enlargement becoming so extensive as to rupture the epidermis. Some hypertrophy of the hypodermis may occur in roots of *Narcissus* following various treatments, but it is always general and never very pronounced; and its occurrence is erratic. A similar condition occurred in the roots of *Allium* (fig. 20).

KRAUS and co-workers (14) have reported that in stems of Red Kidney bean treated with 3% indoleacetic acid in lanolin there is acceleration of mitoses, so that many cells become multinucleate. This condition was not found in root tumors of any of the plants investigated. In many instances, and in all roots studied, there were cells which appeared to be binucleate; but more critical observation showed that the nuclei were on entirely different focal levels, and almost invariably a film of cytoplasm and a cell wall lay between them. Longitudinal sections of roots frequently showed the nucleus of one cell resting against the lower wall, while the nucleus of the cell below lay against the upper wall. If observed in transverse section it might easily be interpreted as a cell containing two nuclei.

The ideas of the relationship between nuclear size and ploidy advanced by BERGER (3) cannot be taken too literally in studying an entire root section, although it probably is true that within the cells of any specific tissue such a rela-

tionship exists. For example, in a transverse section of a root the nuclei of the cortical cells are fairly large and prominent, but nuclei of the cells of the stele may be only one-fourth or one-eighth as large. If the same material is examined in longitudinal section, the nuclei of the cortical cells will be spherical or nearly so, while those in the stele are so elongated in some cases as to appear vermiform. Nuclear volume, however, may be essentially the same.

MARMER (19) has reported that the main or "primary" roots of wheat seedlings treated with indoleacetic, indolebutyric, or indolepropionic acid showed thickening, flattening, curling, and excessive root-hair formation at pH 4.6. In my investigations, root-hair formation was observed only after 120 hours or more in nutrient solution subsequent to treatment. The pH of the nutrient solution was approximately 5.5 at the time the bulbs were placed in it after treatment; and while no attempt was made to confirm this fact, it is probable that the physiological activity of the immersed roots resulted in the shifting of the pH to a point where root-hair formation was encouraged. An investigation of this phase of the work, using controlled pH, might be of interest.

That mitosis appears to be normal in those regions of *Lilium* stems showing response to treatment with indoleacetic has been reported by BEAL (1). He found the diploid chromosome number to be present without exception. This is in agreement with these observations of roots of *Allium*, *Tulipa*, and *Narcissus* treated with indoleacetic acid and other growth substances. In the root primordia formed in *Allium* roots many mitotic figures were observed; and as far as could be determined, all nuclei had the diploid number of chromosomes.

LEVAN found that normal division occurred in most meristematic cells of *Allium*, but that some developments in the enlarged cortical tissues were of particular interest, among these being the occurrence of mitosis as far as 3 mm. from the root apex. LEVAN also found that while cells at the apex showed a normal diploid chromosome number, the majority of those farther back showed tetraploidy, and many of those still farther back showed octoploidy. In *Tulipa*, *Allium*, and *Narcissus*, mitosis occurred as far as 10–16 mm. from the root apex, far beyond the range of the terminal tumor; but no increase in chromosome number was observed in either *Tulipa* or *Narcissus*. Cells with 3n and 4n chromosomes were observed in *Allium*, but the number was sufficiently small to lie within the range of the normal occurrence of polyploid cells. As stated before, mitosis in the enlarged cortical cells was exceedingly rare.

Summary

1. Bulbs of *Allium cepa*, *Tulipa* (vars. John Ruskin and Louis XIV), and *Narcissus* (var. Paper White) were rooted and treated with dilute solutions of various growth-regulating substances.

2. Roots of *Allium* and *Narcissus* responded quickly to treatment with all substances except tryptophane by the formation of more-or-less globular terminal tumors. Tumors induced by tryptophane generally were longer, more slender, and somewhat more spindle-shaped. Roots of *Tulipa* showed little enlargement. Root-tip enlargement, where present, was due to an increase in the size of the cortical cells and not to an increase in the number of their rows.

3. Treatment resulted in the slowing down or complete cessation of root elongation. In some cases the effect was only

temporary, but in others it was permanent.

4. Histological responses occurred first in the meristematic region, chiefly in the cortex, where cells underwent division in all planes.

5. Division in the enlarged cortical cells was rare; but mature cortical cells above the tumors and as far as 16 mm. above the root apex were stimulated to divide. Such divisions generally were typical and in a transverse plane.

6. Pericyclic cells first responded by transverse divisions, forming rows of isodiametric cells with dense cytoplasm and large nuclei. These cells later divided radially and tangentially, resulting in considerable proliferation.

7. Proliferation did not occur in roots of *Narcissus*. Radial and tangential divisions occurred in the endodermal cells, but no true proliferation was apparent.

8. Proliferation of pericycle and development of numerous root primordia occurred in roots of *Allium*. Proliferations also occurred in both varieties of *Tulipa*, but the formation of definite, organized root primordia was not observed.

9. A few polyploid cells were seen in *Allium* roots, but none was found in either *Tulipa* or *Narcissus*.

10. Binucleate or multinucleate cells were not observed in any root subjected to any treatment.

The writer acknowledges with sincere thanks the advice and aid given by Dr. J. M. Beal of the University of Chicago during the course of this investigation.

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ENHANCED AUXINIC ACTIVITY OF TOMATO TISSUES IN PRESENCE OF L-TRYPTOPHANE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 558

GEORGE K. K. LINK AND VIRGINIA EGGERS

By means of procedures reported earlier (3, 4, 8), the effect of the addition of l-tryptophane[2] on the auxinic activity of lyophilized and fresh tomato tissues was tested. Extracts of tomato hypocotyls were used as controls and compared with extracts of hypocotyls inoculated with *Phytomonas tumefaciens*.

Dry weights of both control and inoculated hypocotyls (table 1) equal about 10% of their wet weights. Auxinic activity of the residue of each sample is expressed in an arbitrary unit, calculated on the basis of 1 gm. dry weight in 1 cc. of agar, and an average sensitivity of *Avena* coleoptiles of 16° curvature for 30 γ indoleacetic acid per liter of agar (3, 4).

TABLE 1

SAMPLES USED IN EXPERIMENTS 1–4 AND HISTORY OF THEIR SOURCES

Seed planted	Hypocotyls inoculated	Hypocotyls collected, lyophilized, and powdered	Hypocotyls collected and used fresh
3/7/43	4/8/43	5/7/43 (Exp. 2)	.....
5/8/43	6/1/43	6/28/43 (Exps. 1, 3)	.....
8/24/43	9/14/43	.....	10/6/43 (Exp. 4)

Procedures used in experiments 1–4 are given in the legends of the tables.

An identical experiment was carried out with powder of the 5/4/43 lot. Parallel results were obtained, but the auxinic activity of these samples was greater, showing that the cultural and nutritional histories of the plant affect the auxinic activity of its extracts.

The results of experiment 4 (table 5) corroborate earlier findings (3, 4) that extracts of crown-gall tissues are more active auxinically than those of healthy tomato tissues. They also show that, while extracts of lyophilized tissues are not so active as those of fresh tissues, the findings for lyophilized tissues are applicable to fresh tissues (3).

EXPERIMENT 1: TABLE 2

AUXINIC ACTIVITY, IN UNITS, OF (1) RESIDUES OF EXTRACTS A, B, C OF LYOPHILIZED POWDER (6/28/43) IN 200 CC. OF ETHER, AND OF (2) 1 MG. OF TRYPTOPHANE (D) DISSOLVED IN 200 CC. OF ETHER AFTER STANDING IN THE DARK AT 4° C. FOR 7 DAYS. RESIDUES TAKEN UP IN AGAR AT 60° C.

Source of residue	Activity
a. 0.4 gm. powder	108.0
b. 0.4 gm. powder, plus 1 mg. tryptophane added to residue before taking up in agar	129.6
c. 0.4 gm. powder and 1 mg. tryptophane placed simultaneously in ether	662.4
d. 1.0 mg. tryptophane in 200 cc. ether	4.8

The findings of experiment 1 (table 2d) indicate either that the tryptophane used contained some auxinically active impurities or that a slight amount of it is converted into active material by aqueous ether or in the course of being taken up in warm agar (1, 7). The latter is indicated, since experiment 1 (table 2b) shows an increase in auxinic activity when tryptophane is added to warm agar containing residues of plant extracts. This, together with the general data of all experiments, indicates that results obtained by the standard methods of extraction and of taking up of residues in

[1] This work was supported in part by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2] We are indebted to our colleague, Professor EARL EVANS of the Department of Biochemistry, for a gift of l-tryptophane prepared in his laboratory. Eastman's l-tryptophane was also used.

warm agar are measures, not only of the auxinically active substances present in the sample at the onset of extraction but also of the auxinically active materials obtained from tryptophane by conversion. Results of experiment 3 (table 4) show that time is a factor in the conversion of precursors to active material,

EXPERIMENT 2: TABLE 3

AUXINIC ACTIVITY, IN UNITS, OF (1) RESIDUES OF EXTRACTS A, B, C, D OF LYOPHILIZED POWDER (5/4/43) IN 100 CC. OF ETHER AFTER STANDING IN DARK FOR 12 HOURS, TAKEN UP IN AGAR AT 60° C., AND OF (2) AGAR BLOCKS, 6 CU. MM., AFTER STANDING 2 HOURS IN 0.132 CC. H_2O CONTAINING 2 MG. TRYPTOPHANE (E)

Source of residue	Activity
a. 0.2 gm. powder	165.0
b. 0.2 gm. powder boiled 15 minutes in 10 cc. H_2O prior to placing in ether, plus residue of ether extract of H_2O used in boiling the powder.*	85.8
c. 0.2 gm. powder, plus 0.5 mg. tryptophane placed in ether with the powder	176.0
d. 0.2 gm. powder, boiled 15 minutes in 10 cc. H_2O prior to placing in ether, plus 0.5 mg. tryptophane placed in ether with the powder, plus residue of ether extract of H_2O used in boiling the powder*	67.65
e. 2.0 mg. tryptophane dissolved in 0.132 cc. cold H_2O and taken up by 12 cubes of agar, each 6 cu. mm.	0.0

*In similar tests, residue of ether extract of this water generally is auxinically negative.

and that the activity obtained at any moment is not an index of a definite fraction of the total potential auxinic activity of the sample.

The results of experiment 2 (table 3e) indicate that tryptophane either does not penetrate the epidermis of the upper part of the *Avena* coleoptile or is not auxinic for its tissues. They may indicate either that these tissues, unlike those of tomato, and possibly of bean (2), are not able to bring about conversion of tryptophane, or that the 100 minutes during which the agar blocks are in contact with the tissues of the coleoptile are not sufficient time for a measurable conversion.

EXPERIMENT 3: TABLE 4

AUXINIC ACTIVITY, IN UNITS, OF RESIDUES OF EXTRACTS OF 0.4 GM. LYOPHILIZED POWDER (6/28/43), WITH AND WITHOUT 1 MG. TRYPTOPHANE, IN 200 CC. ETHER, AFTER STANDING IN DARK AT 4° C. FOR 12 HOURS, 6 DAYS, AND 13½ DAYS. RESIDUES TAKEN UP IN AGAR AT 60° C.

Extraction time	Activity	
	Without tryptophane	With tryptophane
12 hours	54.0	85.0
6 days	60.0	640.0
7 days	20.0	910.0
Total 13½ days	134.0	1635.0

EXPERIMENT 4: TABLE 5

AUXINIC ACTIVITY, IN UNITS, OF RESIDUES OF EXTRACTS OF 5.0 GM. OF FRESH TOMATO HYPOCOTYL AND OF INOCULATED HYPOCOTYLS (10/6/43), SLICED 1–2 MM. THICK, PLACED IN 50 CC. ETHER, WITH AND WITHOUT 1 MG. TRYPTOPHANE, AFTER STANDING IN DARK AT 4° C. FOR 12 HOURS. RESIDUES TAKEN UP IN AGAR AT 60° C.

	Noninoculated hypocotyl		Inoculated hypocotyl	
	Without added tryptophane	With added tryptophane	Without added tryptophane	With added tryptophane
Activity	32.3	256.0	836.9	1390.7

The findings of experiment 2 (table 3a–d) indicate that the conversion of tryptophane is brought about by enzymes of the tomato tissues. Tryptophane apparently is a possible precursor of auxinically active materials (5, 7).[3]

[3] A recent paper indicates that tryptophane is not the precursor of "auxin" in corn and wheat

The rate and amount of conversion during extraction is one limiting factor in the activity obtained. Any one of the steps whereby plant proteins are converted into polypeptides, polypeptides into tryptophane, and tryptophane into active material (**1, 3, 5, 6, 7, 9**), as well as the rate at which tryptophane is synthesized in the plant, may be limiting as to the amount of active material present at any moment.

Accordingly, results obtained with the coleoptile test are indirectly, in part at least, an index either of the rate of the activity of these enzymes or of the concentrations of the enzymes involved, or of both. At any moment, therefore, crown-gall tissue either contains more of these enzymes than do normal tissues, or its enzymes are more active. Extractions extending through 2 years often do not exhaust the precursors of the active materials of these tissues, showing that generally amounts of the precursor materials are not the limiting factors.

So far as understanding regulation of growth by auxinically active substances is concerned, the critical problem deals not merely with the concentration of these substances and of their precursors. The heart of the problem is this: that in the presence of an excess of precursor, and with the proper enzymes actually or potentially available, conversion at any moment proceeds at a rate to yield that concentration of active substances which effects the amount and kind of growth and development realized. Auxinically active substances probably are agents of growth regulation, but the regulation itself apparently is that event in the plant which determines the time and place of appearance of these agents as well as their concentration.

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FREQUENCY OF POLYEMBRYONY IN FRAXINUS SEEDS

G. P. STEINBAUER

During the course of some experiments on delayed germination of *Fraxinus* seeds (1), it was noted that the individual samaras occasionally gave rise to two or more seedlings. At first it was thought that this might be accounted for by the presence of more than one seed per fruit. Closer examination indicated that while this was usually true, there were instances in which twin embryos developed from an individual seed. Polyembryony is infrequent in angiosperms, although it may be common in certain groups, such as *Citrus* and *Poa*.

Table 1 indicates the frequency of the phenomenon as observed in certain lots of *Fraxinus* seeds. The data are based on soaked samaras of *F. americana* L., *F. pennsylvanica* Marsh., *F. nigra* Marsh., and *F. velutina* Torr. var. *toumeyi* (Britt.).

The samara of *Fraxinus* is typically a one-seeded fruit with one embryo per seed. The frequency of two seeds per fruit varied from 0.71 per cent in *F. nigra* to 7.00 per cent in *F. velutina*.

The exact origin of the plural embryos is not known. Since many of the reported instances are based on seedling production, the origin of the multiple embryos is often obscure. WEBBER (3) points out that since neither the production of plural eggs nor the liberation of multiple sperms in the megagametophyte is characteristic of angiosperms, it is doubtful whether simple polyembryony occurs. It is suggested that the more likely origin of multiple embryos is from synergids or antipodal cells. Although most of the twin embryos in *Fraxinus* lie parallel to the long axis of the seed, with radicles near the micropyle, in several instances the embryos were oriented opposite each other, one with the radicle adjacent to the micropyle and the other with it at the antipodal end of the megagametophyte. This may suggest the origin of extra embryos from antipodal cells, although their possible origin from supernumerary eggs, from synergids, or from sporophytic budding is as likely.

Polyembryony may occur in *F. pennsylvanica* despite the absence of an observed case. In several plant groups there is a high mortality among multiple embryos during early development. In certain gymnosperms as many as 200 embryos may originate in the seed although only one or a very few survive. TRAUB (2) has shown that the survival of multiple embryos in *Citrus* is dependent not only on the inherent tendencies but also on such factors as available food supply, temperature, etc. It is possible that such factors may also operate to determine embryo survival in *Fraxinus* and other seeds.

TABLE 1

NUMBER OF SEEDS PER FRUIT AND EMBRYOS PER SEED IN FOUR SPECIES OF FRAXINUS

SPECIES	No. OF FRUITS EXAMINED	PERCENTAGE		
		Fruits with 1 seed	Fruits with 2 seeds	Seeds with twin embryos
F. nigra	2773	99.29	0.71	0.11
F. americana	2610	98.90	1.10	0.11
F. pennsylvanica	3754	99.02	0.98	0.00
F. velutina	2293	93.00	7.00	0.13

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CURRENT LITERATURE

Edible Wild Plants of Eastern North America. By MERRITT LYNDON FERNALD and ALFRED CHARLES KINSEY. Cornwall-on-Hudson: Idlewild Press, 1943. Pp. xiv+452. $3.00.

A book such as this is of great value at any time, particularly the present. It is very comprehensive, not only in the great number of forms given, but in the clear, adequate descriptions of them, their habitat and range, and how they may best be utilized. The chapter on Poisonous Flowering Plants Likely to be Mistaken for Edible Species is a valuable section. Like the rest of the book, its statements are positive and clear.

Many are familiar with various wild plants useful as food. Nuts, berries, pot-herbs, and salad plants are common in modern diets, although the degree to which some of them, either directly from the wild or under partial or intensive cultivation, are used, is often not realized. They are now taken for granted. The authors list dozens more which are useful, or could be made so. One is fascinated to turn the pages and find again and again familiar subjects listed among the wild species.

The imagination is stimulated to find suggested purees and soups from elder pith, flowering spikes of cat-tails, daylily buds, and dewberries; starchy foods from lichens, lily bulbs, white waterlily, evening-primrose; cat-tail pollen, clover heads and seeds, inner bark of pine and hemlock and many daily acquaintances; pot-herbs and salad plants of many fairly familiar and less familiar kinds, including clover, chickweed, plantain, mallow, yucca flowers, burdock and Scotch thistle; nibbles and relishes including dried ginger, trailing arbutus flowers, rose petals, alder buds and bark; pickles and condiments in great array; drinks from slippery elm, sassafras, holly leaves, elder flowers, persimmon seeds, dandelion root, chinquapin, and many familiar berries and roots; sugars, confections, preserves from many less usual species and others now taken for granted, such as strawberries, raspberries, cranberries, walnuts, pecans, and many another. In addition, there are lists covering fruits suitable for drying, oils and butters, chewing gums and substitutes, and emergency foods.

Seldom indeed has so comprehensive and authoritative a book dealing with useful wild food plants been based on such outstanding authority.—E. J. KRAUS.

The American Land: Its History and Its Uses. By WILLIAM R. VAN DERSAL. New York: Oxford University Press, 1943. Pp. vii+xvi, 215. $3.75.

The work treats very briefly and in a popular manner many of the uses and abuses of land devoted to the production of food and feed crops. The final chapter offers suggestions for greater conservation of soil resources and possible methods for increasing crop production.

Various crops are mentioned, but treatment is scarcely more than a list of them, except perhaps Indian corn. There are many excellent clear, often striking, photographs of definite value to the casual reader, for whom apparently the work is primarily intended.

The most significant and pertinent statements in the entire book are found in the last paragraph of the author's preface: "Finally, to all those farmers around the country with whom I have talked and through whose eyes I have seen much more than I thought possible, I offer my appreciation of their patience and my admiration for their wisdom. Sometimes I think they know more about America than anyone else."

And plant problems as well.—E. J. KRAUS.

A Dictionary of the Fungi. By G. C. AINSWORTH and G. R. BISBY. Kew: The Imperial Mycological Institute, 1943. Pp. viii+359. $4.60.

An attempt is made to list alphabetically all the generic names of fungi (Eumycetes and Myxothallophyta) that have been in use to the end of 1939, including the systematic position, distribution, and number of species of each genus. There are also short accounts of the chief families, orders, and classes of the fungi and of the bacteria and lichens; definitions of words used in mycology; the common and scientific names of important fungi; and other matters of interest, such as culture methods and media and short biographies of some of the pioneer workers in mycology. Finally, an appendix gives G. W. MARTIN'S *Key to the Families of the Fungi.*

The book represents a tremendous amount of work on the part of the authors, and it is highly recommended to all who are interested in the fungi.—J. M. BEAL.

Diseases and Pests of Ornamental Plants. By BERNARD O. DODGE and HAROLD W. RICKETT. Lancaster: Jaques Cattell Press, 1943. Pp. xi+638. $6.50.

Various treatises have been issued from time to time dealing with the pests which infest various crops of economic importance. The present volume is a comprehensive one concerning ornamental plants. Part I covers the general types of pests which are likely to be encountered, of animal, plant, or undetermined origin, and the principles involved in pest control. Part II is an extensive alphabetical list of ornamental plants, together with the pests affecting each, and methods of control.

The text is direct and clear, the many illustrations well done and to the point. It should prove a useful handbook and manual for those who attempt to grow ornamentals and to those called upon to furnish suggestions on pest control.—E. J. KRAUS.

Frontiers of Cytochemistry: The Physical and Chemical Organization of the Cytoplasm. Edited by

NORMAND L. HOERR. Lancaster: Jaques Cattell Press, 1943. Pp. 334. $3.50.

This volume is number X in a series of Biological Symposia. It is a tribute to the work of Emeritus Professor ROBERT R. BENSLEY, of the University of Chicago. Originally the papers were prepared for the celebration of the seventy-fifth anniversary of Dr. BENSLEY's birth and were presented before an audience of about 500 on November 13, 1942.

A brief appreciation of Dr. BENSLEY as a leader in research, dealing with the physical and chemical organization of protoplasmic constituents, is written by E. V. COWDREY. The published papers number fourteen, the first of which, by ARNOLD LAZAROW, is a summary of the work done in BENSLEY's laboratory during the last ten years. Many features of cellular organization and behavior are discussed in the several papers. The separation of protoplasm into its individual components, and the analysis of these components by physical and chemical means, form the central theme of the symposium. Although most of the work has been done on animal cells, it seems probable that the findings are fundamentally applicable to all protoplasm, with some variability in the quantitative amounts of the constituents present. The last paper in the volume is by Dr. BENSLEY, on the Chemistry of Cytoplasm.

The papers constitute a very valuable summary of progress in a difficult field and emphasize the importance of leadership such as Professor BENSLEY has been able to give in this field. An excellent portrait of Dr BENSLEY is appended.—C. A. SHULL.

Flora of Peru (Leguminosae). By J. F. MACBRIDE. Chicago: Field Mus. Nat. Hist. Bot. Ser. 13, 1943. Part 3, no. 1. Publ. 531. Pp. 1–507.

This is a conventional taxonomic treatment of the single family Leguminosae, with 18 genera of the Mimosoideae, 28 of the Caesalpinioideae, and 72 of the Papilionatae recognized as occurring in Peru. Keys to the genera and to the species are provided, with ample generic descriptions as well as descriptions of all recognized species. The synonymy is given for each species, and under each all Peruvian collections representing the entity are cited. Approximate extra-Peruvian distribution is indicated.—E. D. MERRILL.

Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. New York: Chilean Nitrate Educational Bureau, Inc., 1943. Pp. 92.

The fourth supplement to the third edition of this bibliography continues the plan of arrangements of previous supplements. There are approximately 700 abstracts, dealing with more than half of the known elements and with more than 100 kinds of crop plants. The abstracts are arranged by elements, in alphabetic order, from aluminum to zinc and zirconium, with the rare earths and miscellaneous at the close.

The triple index, by elements, by botanical species, and by authors, leaves nothing to be desired. It is an important work, and a service which deserves the wholehearted appreciation of American scientists. It is especially useful to those who are engaged in mineral nutrition investigations.—C. A. SHULL.

The Food Resources of Africa. By THOMAS S. GITHENS and CARROLL E. WOOD, Jr. Philadelphia: University of Pennsylvania Press, 1943. Pp. 105. $1.50.

This brief treatise gives a general survey of Africa's agricultural resources. Based on the continent as a whole, statistics of production are given for its major geographical—and to a lesser extent its ecological—areas. These form a brief crop index of present production, utilization, and export of the principal food crops.—E. J. KRAUS.